皮膚科及泌尿器科雜誌 第二十一卷 第三號 大正十年(一九二一年)三月

論說

滿洲ノ白癬

Dr. M. Ôta: Recherches sur les trichophyties en Mandchourie.

醫學士 太田正雄

目次

緒論。各論。(第一類大芽胞菌)岩穴狀白癬菌 尖圭狀白癬菌 紫色白癬菌 禿滑狀白癬菌 星芒狀石膏樣白癬菌 雪狀白癬菌 白色黃癬樣白癬菌 衣嚢樣白癬菌 (第二類)一種ノ小芽胞菌 (第三類)レピデルモフィートン・ナンギナール 附圖說明。引用書目。附表

緒論

本研究ハ著者ガ奉天南滿醫學堂ニ勤務中大正八年二月ヨリ本年(大正九年)六月ニ亙ッテ行ッタモノデアル此材料トシテハ同學堂皮膚科教室外來ニ來レル病例竝ニ著者ガ同地附近ノ回々教徒部落ニ趣イテ得タル病例ヲ以テ之ニ當テタ其間本年一月七日ニハ南滿醫學會ニ於テ又三月二十九日ニハ金澤ニ開催セラレタル日本皮膚科學會ニ於テ其內容ノ大體ヲ演述シ竝ニ菌培養ニ就テ實物說明ヲナシタ尤モ其等ノ時期ニ陳述シタル事項中本論ニ於テ訂正シタ所モ尠クナイ著者ハ今ヤ南滿醫學堂ヲ辭セントスルニ當リ取急イデ本稿ヲ起シタガ此時ニ於テ常ニ著者ノ爲メニ多大ノ盡力ヲナサレタル我教室醫員高橋晃・王佩韋・郭鐘名・佐藤昌一・杉本大周ノ諸君ニ對シテ感謝ノ意ヲ表スルノデアル

✓

OTA, M. 1921

Recherches sur les trichophyties en Mandchourie.

J. Jap. Derm. Syph

Jap. (Zeit). Derm. Urol., 21, 201. 330. 340.
2

To be microfilmed.

是等ノ關係ヲ表記スレバ次ノ如クデアル

菌種 ＼ 症候	頭部白癬	小水疱性斑狀白癬	頑癬ハタケ	爪甲白癬	汗疱樣白癬
Tr. crateriforme		1*			
Tr. acuminatum		4*	2		1
Tr. violaceum	2	1			
Tr. glabrum				2	
Tr. gyps. asteroides	4	2			
Tr. niveum					2
Tr. favif. album	1				
Tr. plicatile (?)	1				
Une espèce de Microsporum	17	1	1		
l'Epidermophyton inguin.		1*			

* 右表ノ斑狀白癬中股間又ハ陰嚢ニ生ジテ頑癬ノ初期又ハ其輕症ト看做スベキモノ岩穴狀菌ニ於テ一・尖圭狀菌ニ於テ一・エピデルモフィートンニ於テ一デアル

菌種鑑別上ノ困難　著者ハ右ノ菌種ヲ鑑別スルニ當リ屢〻困難ヲ感ジタ第一サブロー氏ガ milieu international d'epreuve トシテ使用スル培養基ノ材料タルベキシャニューノ粗製マルトーズ同塊狀葡萄糖及ビシャッサンノ顆粒狀ペプトン等ヲ手ニスルコトガ出來ナカッタコトデアル是等ノモノハ著者ハ日本製品ヲ以テ之ニ代ヘ別ニ麴汁寒天マルト汁寒天等ヲ用キタ

次ニハ髮内菌・髮内外菌・髮外菌ノ區別デアル其内純髮内菌ダケハ（是レハ唯動物實驗上得タモノデ患者ノ病毛デハ著者ハ一度モ此例ニ遭遇シナカッタ）慥カニ之ヲ認知スルコトヲ得タガ髮外菌ト髮内外菌トハ其區別甚ダ困難デアル而モサブロー氏自身ガ髮外性トスル菌ガ往々髮内外性トシテ現ハレルコトノアルヲ認メテ居ル（例ヘバ黃癬樣菌・石膏

偖標題ニハ滿洲ノ白癬ト記シタガソレハ主トシテ奉天ヲ中心トシタモノデアル其他ニハ唯僅カノ例ガ鐵嶺・本溪湖等ノ沿線カラ得ラレタ

本論ニ於テ取扱ッタ症例(是レハ菌培養ニ成功シタモノ、ミヲ集メタノデアル)ハ凡テヾ四十三例デアッテ症候ハ頭部白癬・小水疱性白癬・斑狀鱗屑性白癬(ハタケ)・頑癬及ビ爪甲白癬デアル其他「汗疱ニ就テ」ノ第二回報告ニ漏レタル汗疱様外觀ヲ有スル手掌及足蹠等ノ白癬ノ數例ヲモ亦序ニ本論中ニ收メタ

症候ノ分類中陰囊・股間等ニ生ズル斑狀白癬ハ後來蔓延持久甚シキニ亙ラバヘブラ氏典型ノ頑癬トナルベキモノモ其ノカクノ如クニ至ラザル間ハ之ト區別シテ斑狀白癬ノ部ニ收メタ而シテ著者ノ場合ニ於テハ其病原菌ハ必シモエピデルモフィートンノ一種ニ止マラズ岩穴狀菌殊ニ尖圭狀菌モサウデアッタ

次ニ菌種ノ分類法デアルガ著者ハサブロー氏ノ分類法鑑別法ガ甚ダ完全デアルモノト信ズルコトガ出來ナイ然シナガラ現在ノ狀態ニ於テハ著者ハ大體ニ於テ氏ノ創見ニ據ルヨリ外止ムヲ得ナイコトヲ告白スルモノデアル殊ニ小芽胞菌ト大芽胞菌トノ區別ハサブロー氏ノ說ク所ヲ見テモ甚ダ曖昧タルヲ免レヌ著者ガ本論ニ於テ小芽胞菌種トシテ分類シタモノ、如キハサブロー氏著書ニ於テ之ト同一ナルモノヲ發見スルコトガ出來ナイ故ニ强ヒテ其菌名ヲ既知ノモノニ求メエズ一種ノ小芽胞菌トシテ唯其性狀ヲ詳記スルニ止メタ而モ本菌ニ似タル特徵性狀ヲ有スルモノヲ著者ハサブロー氏ノ大芽胞菌ノ一種ニ於テモ亦小芽胞菌ノ一種ニ於テモ發見スルヤウナ次第デアル畢竟白癬菌ノ屬スル Fungi imperfecti ナル部門ハ現在ニ於テ其研究未ダ甚ダ疎ナルヲ免レナイ而シテ皮膚科ニ於テ重要ナル白癬菌ノ如キ殊ニ其植物學上ノ分類ニ關シテ今後尙ホ專門家ノ研究ヲ待ツコトノ多イモノデアル

症例及ビ菌種ノ關係ニ關スル概括　培養例(卽チ本報告ノ患者例)四十三例中日本人十七人(內女五人)支那人二十三人(內女一人)英人一人(男)露西亞人二人(內女一人)デアル菌種別ニスルト岩穴狀菌一・尖圭狀菌七・菫色菌三・禿滑菌二・星芒狀菌六・雪狀菌二・白色菌一・衣襞狀菌(?)一・一種ノ小芽胞菌(馬小芽胞菌?)一九・エピデルモフィートン一デアル

ペプトンハ照内氏ノペプトン・マルトーズハ大阪林製・葡萄糖ハ大阪石津製デアルガ糖培養基ノペプトン含有量ハ凡テ一%デアッテマルトーズ葡萄糖ハソレゾレ四%デアル孰レモ培養基製造中本來ノ弱酸性ノマヽニシテ之ヲ中和スルコトヲナサナカッタ

麹汁寒天ノ製法ハ麹ヲ約三〇〇瓩取リ少許ノ水ヲ加ヘ攝氏五十五度ニテ半日温メ然ル後之ヲ一時間煮沸シテ濾過シ再ビ卵白ヲ加ヘテ煮テ透明ナラシメ更ニ之ニ水ヲ加ヘテ全量ヲ約一〇〇〇瓩トナシ其比重ハバリング糖比重計ニテ九トナシ反應酸性ノマヽ之ニ寒天ヲ加ヘ固メル

マルトハ其約三五〇瓩ヲ取リ之ヲ粉末ニシ水ヲ加ヘテ全量約一〇〇〇瓩トナシ攝氏六十五度ニテ三時間温メタル上煮沸シバリングニテ九ノ比重ノ所ニテ寒天ヲ加フ右ノ二種ハ孰レモペプトンヲ加ヘナイ

麹汁寒天上ノ菌ノ發育ハ甚ダ良好デアルガマルト液寒天ノ方ハ必シモ然ラズ且培養ノ形ガ甚ダ變化スルソレデ培養ノ形ガ比較的サブロー氏ノ記述ニ近クナルハヤハリ日本製ナガラ葡萄糖ハマルトーズヲ加ヘタペプトン寒天ノ上ニ於ケルモノデアッタ

菌學上ノ檢査ハ凹面載物硝子ノ上ニ葡萄糖寒天ヲ塗ッタ被覆硝子ノ裏向ケニ載セ之ヲペトリ氏シャーレノ濕室中ニ置クトイフ簡便ナ安置培養法ニ由リ併セテ斜面寒天培養カラ一片ヲトリ之レヲ鏡檢シ兩者ヲ比較シタ

菌種 日本ノ文獻ニ擧ゲラレタル既知ノ菌種名ハ上林氏ニ據ルニ尖圭狀白癬菌・菫色菌・レピデルモフィトン(楠氏)・一種ノ石膏樣菌(小池・三島兩氏)デアル上林氏自身ハ石膏樣菌(顆粒狀及ビ乳色ノ二種)脳廻轉樣菌(後ニ亦雪狀菌)ノ四種ヲ追加シテ居ル

然ラバ則チ著者ニ由リテ岩穴狀菌・禿滑菌(Tr. glabrum)・白色黄癬樣菌・衣嚢樣菌ノ五種ガ新ニ日本文ノ文獻ニ追加セラレタワケデアル又石膏樣菌中特ニ星芒狀菌ヲ詳記シタモノハ亦著者ヲ以テ嚆矢トスル

著者ガ「一種ノ小芽胞菌」トシテ記シタモノハ後日親シクサブロー氏培養ノ蒐集ヲ見ソレト異同ヲ比較シタ後ニ或ハ著者ノ新名ヲ附シ或ハ既知ノ菌名ヲ襲フヤウニスル

樣菌ノ如キ)

又著者ガ既述セルガ如ク小芽胞菌ト大芽胞菌トノ區別サヘモ著者ハ亦一本ノ病毛ニ就テハ完全ニ之ヲ識別スルコトガ出來ナカツタ例ヘバ著者ガ一種ノ小芽胞菌トシテ論ジタル一菌種ノ如キモ其病毛ノ芽胞鞘中ニ於ケル其芽胞ノ大サハ概シテ言ヘバ三乃至四ミクロンノ直徑ヲ有スルモノデアツタガ時トシテ(少數ナガラ)五乃至六ミクロンニ達スル直徑ヲ有スルモノモアツテ其斷定ヲ躊躇セシメタ

カクノ如キ關係カラ菌名ヲ查定スル場合ニ第一ニ髮內性デアルヤ否ヤトイフサブロー氏ノ第一次的根據ニ據ツテ之ヲ決定スルコトガ出來ナイデ主トシテ培養ノ外觀・菌學上ノ所見ニ據ツテ之ヲ推斷スルヤウナコトモアツタ

第二ニ其培養ノ外形ガ常ニ一定ノモノデハナイ同時ニ造ツタ培養基ノ上ニ於テスラ時トシテ甚ダ異ル外觀ヲ呈スルコトガアル又サ氏ノ標準培養基ヲ用ヰナカツタセイカサ氏ノ記述通リニ出ナイ場合モアツタ

最後ニ動物試驗モ亦困難ナ仕事デアル石膏樣菌ノ如ク必ズ陽性ニ移植セラルベキモノデモ移植試驗ノ全部ガ成功スルトイフワケニハ行カナカツタ其故成功ノ場合ハ之ヲ陽性ト考ヘテ可ナルモ不成功ノ場合ニハ必シモ之ヲ陰性ト見做スワケニハ行カヌノデアル

サブロー氏ノ記ス白癬菌種ハ多クハ氏ノ始メニ命名スル所ノモノデアル卽チ是等菌種ノ類ヲ分ツコトハ氏ニ取リテハ割合ニ容易ナコトデアツタラウト思フ唯氏ノ記述及ビ其寫眞圖ノミニ依ツテ他國ノ白癬菌種ヲ分ツノハ甚ダ不分明ノコト多ク靴ヲ隔テヽ痒ヲ掻クノ感ニ堪ヘナイ

著者ノ此ニ記ス一種ノ小芽胞菌ノ如キハ滿洲ニ於テハ其蔓延廣ク最モ重要ナル種類デアル而モ著者ハ此種ノ決定ノ爲メ數回逡巡躊躇シタ而モ遂ニサブロー氏ノ菌名ニ其同一ノモノヲ發見スルコトガ出來ナイデ唯一種ノ小芽胞菌トシテ論ズルノ止ムヲ得ザルニ至ツタ

著者ノ用ヰタル培養基　三%ペプトン寒天・葡萄糖ペプトン寒天・マルトーズ・ペプトン寒天及ビ麴汁寒天・マルト汁寒天・馬鈴薯平板等ヲ用ヰタ

變態（プレオモルフイズム）　葡萄糖寒天斜面デハ白色絨毛狀ノ廣イ芝生ヲ、形成シタ其際中央部ハ幾分深クナツテ岩穴狀態ノ形迹ヲ殘ス（插入圖第二圖）

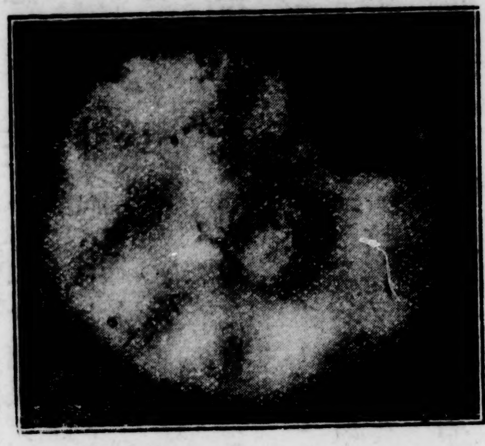


Fig.2 岩穴狀菌ノ Pleomorphismus.

菌學　葡萄糖安置培養法デ觀察スルニ數日ニシテ短大ノ穗狀ノ外芽胞ヲ作ルガ夫レ（第三圖a）ハ多クハ一條ノ枝ノ兩側ニ附著スル然シソンナ hyphes sporifères simples ノ他ニ尙分岐シタル枝ニ葡萄房狀ニ簇生スル芽胞ヲモ見ル（同b）次ニサブロー氏ノ所謂 la migration protoplasmique ガ顯著ニ發現スル（同c）枝ヲ形成スル部ノ原形質ガ吸收セラレテ芽胞群ノミガ枝アル時ノ如ク帶狀ニ列ヲ成シテ殘留スルモノデアル

ソレカラ安置法デハ良ク分ラナカツタガ斜面ノ稍、舊イ培養ヲ取ツテ檢査スルニ菌絲ノ末端又ハソレヨリ分岐シタ側枝ノ末端ガ紡錘狀又ハ棍棒狀ニ膨脹シ時トスルト其內ニ一箇又ハ數箇ノ原形質ノ塊ヲ藏シテ居ル（les extrémités massuées）是レニハ單細胞ノモノト數箇細胞ヨリ成ルモノトガアル（插圖第四圖a・b）或ハ亦ソウ云フ太イ末端カラ芽胞或ハクラミドスポールノ派出スルモノガアル（c）同ジク舊イ斜面培養ノモノニハ毛髮又ハ鱗屑中ニアル時ノヤウニ短大ニシテ著明ノ二重輪廓ヲ呈スル菌絲ガ見ラレルノデアル（d）

動物試驗　著者ノ場合ニ於テハ再三回反復シタガ陰性デアツタ

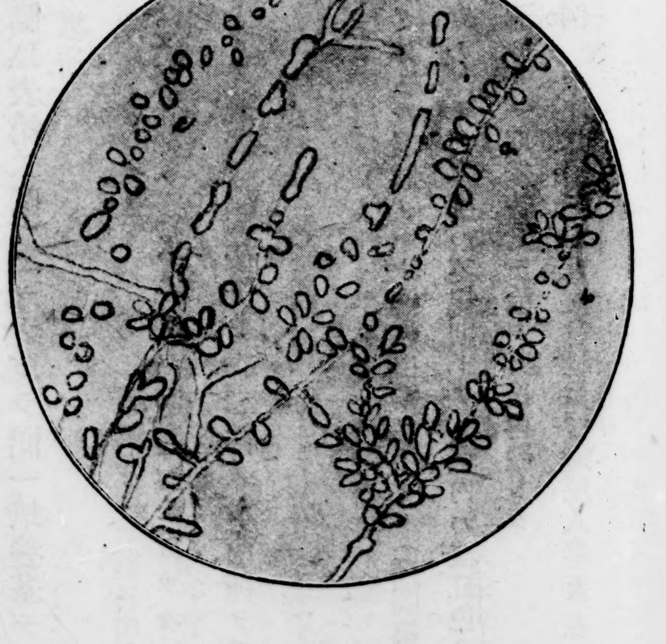


Fig. 3 Tr. craterif. 葡萄糖安置培養第七日

Fig.4 Tr. craterif. 葡萄糖寒天斜面第三十二日培養
（メチレンブラウ染色）

ソレ故本菌ガ確カニ髮內菌デアルトイフコトヲ證スルコトハ出來ナカツタ然シナガラ上述ノ培養上菌學上ノ所見カラ之ヲ岩穴

各論

第一類 大芽胞菌種

第一種 岩穴狀白癬菌 Tr. crateriforme Sabouraud.

サブロー氏ニ據ルニ巴里デハ髮內菌ガ多數ニ存在シ本種ノ如キ其重要ナル種類デアルト云フガ日本デハ著者ノ寡聞ナル未ダ此菌ノ發見セラレタノヲ知ラヌ著者ノ場合ニ於テモ本種ハ唯一例ニ於テ得ラレ而シテ患者ハ英國人デアツタ

臨牀的所見 第一例バンケー三十二歲商人男(奉天在住英國人)

診斷・陰囊及股間ノ鱗屑性斑狀白癬大正八年十月二十三日初診

本例ハ十月中旬ヨリ發生シ現在ハ兩側ノ鼠蹊下部及ビ陰囊ニ爪甲大ノ斑散在ス其色ハ帶黃色デ其輪廓ハ不規則ナガラ判明デアル但シ健康面ヨリ高ク隆起シテ居ナイ表面ニハ小鱗屑ヲ頂クガ水疱ヲ見ナイソレデ外觀ガジベール氏薔薇色粃糠疹ニ似テ居ル此紅斑以外ノ皮膚モ往々輕度ノ濕疹狀態ヲ呈シテ落屑ガアル是クノ如キモ亦頑癬ト名稱スベキデアルカ恐ラク之ヲ放置スレバ典型的ヘブラ氏型ノ頑癬トナルダラウト思フ

鏡檢 紅斑部ノ鱗屑中ニ無數ノ菌絲ガアリ其幅員ハ三ミクロン強デ兩緣ハ平行デ節ヲ見ナイ又原形質中ニ顆粒ガナイ稀ニ菌絲中ニクラミドスポオルヲ見ル(插入圖第一圖)

培養 鱗屑移植部ヨリ四五日ニ堅ク且乾燥シタ外觀ヲ呈スル白色ノ小點ヲ生ジ十數日ヲ經テ中央部ノ陷落ヲ來ス (附圖IX表第二圖ハ葡萄糖寒天ニ於ケル第二十日ノ原發培養ヲ示ス)

巨大培養デハ四%マルトーズ寒天四%葡萄糖寒天孰レモ大同小異ノ外觀デアル充分發育シタモノデハ其直徑ハ三乃至四糎位アル純白色デ脆弱デ陷沒部ニハ小龜裂ヲ作ル裏面ハ黃褐色ヲ呈ス

Fig.1 鱗屑中ノ Tr. crateriforme ノ菌絲

ル但シ數代移植ヲ行フニ必シモ原型ノ如クハナラズ或者ハ純白色ナラズ寧ロ微灰褐色デ周圍ガ岩山ノヤウニ聳立シテ中央ノ深イ陷凹部ヲ圍ミ而シテ陷凹部ニハ漸次著シキ龜裂ノ生ズルヤウナノモアル或ハ隆起セル外輪ヨリ外方ニ更ニ平坦ナル粉狀ノ芝生ヲ作ルモノモアル

馬鈴薯上ニハ帶褐灰白色ノギザギザシタ山形ヲ作ル周邊ハ圓ク光澤ハ乾燥又ソノ表面ニ白色ノ絨毛ヲ發生スル所モアル

十二月九日診

一ケ月許前ニ項部ニ濕布ヲ置イタ所ガ約十日前カラソコニ二錢銅貨大ノ二箇ノ紅斑ヲ生ジ其周圍ニ小水疱ガ環狀ニ竝列シテ生ジタ尙ホ二三日前ヨリ陰股部ニモ瘙痒アル環狀疹ガ生ジタ

第四例　川內某女大正八年八月生千葉縣出生奉天在住大正八年十二月九日診

左腋窩ヨリ肩胛部ニ掛ケテ二錢銅貨大ノ圓形病竈ガアツテ小水疱性ノ發疹ガ略〻三列ノ同心性環ヲナシテ居ル四五日前ヨリノ發病ニ係ル患者ハ四月中內地ヨリ來タトイフカラ滿洲デノ感染デアル

第五例　藤竿某男三十三歲銀行員大分縣人開原在住大正八年七月九日診

尿道炎ノ爲メニ入院シテ居タガ八月上旬ニ至ツテ兩股間ニ數箇ノ斑狀ノ白癬病竈ヲ生ジタ此場合小水疱ノ形成ハ著シクナク爪甲大デ褐黃色ヲ呈シ且ツ落屑セル斑紋ヲ以テ主徵候トナシタヘブラ氏頑癬ノ典型トハ異ルガ又假ニ此內ニ編入スル

(乙)頑癬型

第六例　大塚某男三十六歲商愛媛縣人奉天在住大正八年六月十八日診

慢性尿道炎ニテ入院中兩股間ニ慢性濕疹狀ノ頑癬ヲ有スルコトヲ發見シ七月三十一日培養ヲ行ツタ

第七例　チナブ三十歲露西亞商人大正八年十二月一日診

後部尿道炎ニ兼ヲテ數年來治癒セザル頑癬ヲ有ス部位ハ陰部及ビ兩側ノ大腿デ其陰囊ニ於ケルモノハ慢性濕疹ノ形ヲナシ浸潤强ク其境界ハ不判明デアルガ兩側ノ大腿內面ニ於テハ數箇ノ孤立セル圓板狀紅斑ガアリ鱗屑ヲ戴イテ居ル是レハ一ケ月半許前カラ新生シタモノデ漸次增大ノ徵ガアル

(丙)汗疱樣型

第八例　津川某男二十歲愛知縣人奉天在住大正九年五月十七日診

本症ハ本年三月ノ初發ニ係ル始メハ兩側足趾間ノ汗疱樣濕疹ノ狀態ヲ呈シ來リ後ニハ右側第二指ニモ傳染シタ又二週間前ヨリハ右側足趾ノ蜂窠織炎ヲ來シタ

尙ホ本例ニ於テハ右側第二指ニ於テ爪甲白癬モ存シテ居タガ此方ノ菌檢索及ビ培養ヲバ閑却シタ多分同一種ニ由ルモノデアラウト思フ

鏡檢　右ノウチ第二例ノ平見某ヨリ得タル鱗屑ニ就テ見ルト三樣ノ要素ガ見ラレル第一ハ細長イ節ノ長イ菌絲デ其幅ハ二ミクロン强デアリ透明デ顆粒ハ殆ドナク數々Y狀ニ分岐スル第二ハソレヨリ廣ク幅員四ミクロン弱ヲ算フル物デ甚ダ短節デアル或

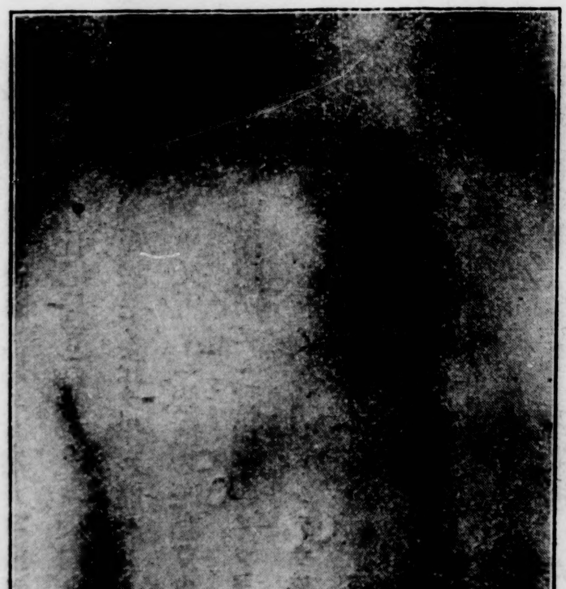


Fig. 5 平見某男 Tr. acuminatum. ニ因ル小水疱性斑狀白癬

狀菌デアルト斷定スルモノデアル

第二種　尖圭狀白癬菌 Tr. acuminatum Sabouraud.

嘗テ著者ガ「汗疱ニ就テ」ノ第二報告ヲ書イタ當座ハ此菌種ニ二型ガ存シテ居ルヤウニ解シタ其內黃褐色濕潤性ノ表面ヲナス甲型ハ或ハ楠氏ノ所謂「結節狀白癬菌ニシテ黃色塊狀ノ菌苔ヲ生ズルモノ」ト同一種デハナカラウカト考ヘタ然シ其後ノ研究ニ由ッテ此レガ僅カニ Tr. glabrum Sabouraud デアルトイフコトガ判明シ既ニ送ル所ノ原稿ノ一部ノ訂正ヲ申込ンダ爲メニ同論文ノ「尖圭狀菌」ノ項ガ稍〻曖昧ナモノニナッタ〔本誌第二十卷第六號ノ二十七頁後ヨリ四行ニ突然 Tr. glabrum トイフ名ノ出テ來ルノハ始メ甲型トナシタモノヲ Tr. glabrum ニ訂正シタノミデ前後ノ關係ヲ顧慮スルニ及バナカッタ結果デアルソレ故「著者ノ Tr. glabrum 型ニモ紡錘狀芽胞ハ出ナイ」トイフ一句ハ「著者ノ場合ニモ紡錘狀芽胞ハ出ナイ」ト改メル又同二十六頁最後ノ行ノ爪甲又無毛部ニ於ケル疾患云々ノ句ヨリ爪甲又ノ三字ヲ削ル〕

上述ノ如キ關係デ今ヤ著者ノ尖圭狀白癬菌ハ孰モ同一ノ種類デソノ異型ヲ擧ゲル必要ガナクナッタソシテ其培養基ハサ氏ノ標準基ヲ用キナイデモカナリ良ク典型的形像ヲ現ハス（附圖IX表第二圖）然シナガラ同一培養基デモ甚ダシク其外觀ヲ異ニスル培養ヲ形作ルコトガアッタ

著者ハ本種ヲ左記七人ノ患者カラ得タ孰レモ無毛部白癬デ毛髮ヲ侵シタモノニハ一例モ遭遇シナカッタ但シ前報告後汗疱樣疾患カラ更ニ新シキ一例ノ本種ヲ得タカラ汗疱ノ原因菌トシテ著者ハ前後併セテ二株ノ本菌種ヲ得タワケデアル

臨牀的所見　（甲）**小水疱性斑狀白癬**

第二例　平見某男二十歲驛員奉天在住大正八年九月十一日診

既往症及現症　右ハ胸膜炎ノ爲メニ內科ニ入院シ連日胸部ニ濕布ヲ卷イタ所ガ著者ノ初診時ヨリ四五日前カラ左肩胛骨下部ニ十餘ヲ算スル環狀ノ小水疱性白癬ヲ生ジタ（插圖第五圖）環ニハ大小ガアッテ其輪廓ハ不規則デアリ環內ハ淡紅色ヲ呈シテ鱗屑ヲ附著スル一度爹硫膏デ治シ其後再發シタ

第三例　永江某男三十五歲會社員愛媛縣人奉天在住大正八年

チ平見・川内・津川種デ就中平見種ハマルトーズ寒天ノマトラニ培養シタノデアルガ約二ケ月デ四糎ノ直徑ヲ有スル圓板トナリ其中央ガ尖圭狀ニ尖ツテ居ルソコヨリハ周圍ニ向ツテ十數條ノ深イ溝ヲ出シ尚ホ同心性ノ淺イ溝モアル色ハ幾分黃褐味ヲ呈シタ白色デ乾燥シ表面ハ粉狀デ又所々ニ極メテ短イ絨毛デ被ハレテ居ル(附圖第IX表第三圖)然ルニ同株菌ノ培養デモ四%葡萄糖寒天ノマトラニ行ツタ或モノニ於テハ同ジク附圖IX表ノ第四圖ニ示シタ如ク幾分ノ差異ガアル卽チ色ハ純白デ乾燥シタ外觀ヲ呈スル圓板デアルガソレデモ尚ホ中央部ニハ丘狀隆起及ビ放射狀溝ノ傾向ハ見エルノデアル插圖第九圖ニ示シタチナブ種モ略、同樣デアル但シ其尖圭狀隆起ハ極メテ低イ

又藤竿種ノ巨大培養デハ中央ニ小結節ヲ有スル丘狀隆起ノ大圓板ヲ作リ十數箇ノ放射狀溝モアルガ其隆起ハ低ク溝モ淺ク且其周緣部ハ中央部ト劃然タル境界ヲナシ淺紫色ニ染マツタ圓輪ヲ作ツタ(插入寫眞圖第八圖)又嘗ツテ著者ハ「汗疱」論ノ第二報告ニ於テカナリ特殊ノ外觀ヲ呈シテ且著シク紫色々素ヲ生ジタ尖圭種ノ一標本ヲ紹介シタコトガアツタ

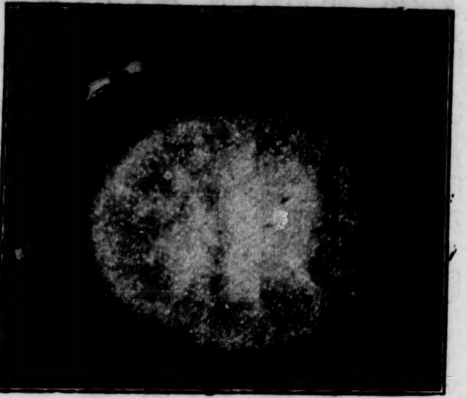


Fig.8 藤竿種マルトーゼ寒天第二十七日培養

カク巨大培養ニ於テ各菌株ノ間ニ多少ノ差異ハアルガ大體尖圭狀ニナル傾向ガアリ且ペプトン寒天ノ上ニハ必ズ尖圭狀ガ著シイ等ノ點カラ竝ニ菌學上動物試驗上ノ點カラ皆同一種デアルコトヲ判定スルコトガ出來ル

Fig.9 チナブ種葡萄糖寒天二十三日培養

ソレデ今度ハ三%ペプトン寒天上ノ培養ノ狀態デアルガ此場合ニハ菌株ノ色ハ純白色デナク帶灰褐ノクレエム色デアツテ光澤モ乾燥樣デナクテ濕潤性デアル又放射狀溝ハ著シクナク寧ロ不規則ノ凸凹ヲ生ズル是等ノコトハサブロー氏ガ其著書ニ記スガ如クデアル殊ニ斜面培養デ基底ノ凝固水ニ近イ所デハ尖圭狀隆起ガ殊ニ著シク且其中心モ小結節ヲ生ジソレカラ棘狀絨毛

Fig. 10 (1)永江種(ペプトン寒天十八日目)
(2)チナブ種(同十八日目)
(3)平見種(ペプトン寒天)
(4)藤竿種(葡萄糖寒天)
(5)永江種(マルトーゼ寒天)

ハ平行セル長イ菌絲ノ膜ノ内ニ分金狀ノ原形質ガ櫛比竝列シテ居ルト見做シタ方ガ適當デアルカモ知レヌ第三ハソウ云フ原形質ガ孤立シテ小判狀ヲナシ且長軸ノ方向ニ竝列スル芽胞連鎖トナッタモノデアル（插入圖第六圖）

培養　本邦ニ於テ尖圭狀菌（同氏ノ命名ニ從ヘバ結節狀菌）ニ就テ最モ詳細ニ記述セラレタルハ楠氏デアルガ氏ハ同種ヲ二乃至三型ニ區別セラレタ然ルニ著者ノ場合ニ於テハ大體マヅ一型デアル唯培養基ノ如何ニ由ッテ幾分外觀ノ差異ヲ見ルノデアル

Fig.6 平見氏鱗屑中ノ絲菌（Tr. acum.）

斜面培養ニ於テハ其種類ヲ問ハズ最初鱗屑移植後四五日ニシテ白色ノ小點ヲ生ジ日ヲ經テソレガ多少絨毛狀トナルガ其際周圍ニ擴マル傾向ヨリモ高ク隆起スル傾向ノ方ガ著シクナル（插入寫眞圖第七圖大塚氏菌葡萄糖寒天第十三日）此傾向ハ聚落ガ大キクナッテモ同樣デアルガ大キクナルニ伴ッテ同心性竝ニ放射狀ノ不規則ナ溝ガ出來テ來ル此際聚落ノ色ハ純白デヤ、乾燥ノ外觀ヲ呈シ幾分硬固ニ見エル然ルニ或種ニ於テハ（藤竿種・チナブ種又汗疱ヅ石尾種等）ソンナニ硬固ノ感ジヲ與ヘズニ眞白ナ如何ニモ軟イ短絨毛ノ丘ヲ作ル而モソノ丘ヲ繞ッテ細イ微黃色ノ輪ガ見エタリ又所々ニ紫色ノ斑點ガ出來ル聚落ノ裏面ハ前型ニアッテハ黃褐色デアルガ後型ニアッテハ黃褐色ノ間ニ紅紫色ノ斑點ヲ現ハスノデアル孰レニシテモ著者ノ場合デハサブロー氏ノ謂フガ如キ中心部ノ指狀毛突起 digitations poilues ハ每常必ズ存スルト云フワケデハナカッタ唯三%ペプトン寒天斜面ノ場合多ク之ヲ見タノデアッタ

Fig.7 大塚種葡萄糖斜面第十三日

葡萄糖又ハマルトーゼ寒天ニ在ッテ培養ガ舊クナッタリ又代ガ重ナッタリスルト聚落ガ尖圭狀ヲ保タナイデ白絨毛狀ノ平面（尤モ緩カナ丘狀ヲナス）ヲナスコトガアル其際往々紫色ノ色素ガ出ルソシテ聚落ノ裏面ハ多クハ紫褐色デアルカウ云フ狀態ハ楠氏ノ白色絨毛狀ノ菌苔ヲ生ジ其基底ニ赤褐色ヲ帶ブルニ至ルトイフ記載ニ一致スル（插入寫眞圖第十圖4・5）然シナガラ糖ヲ加ヘザル三%ペプトン寒天デハコンナ絨毛狀ノ扁平ナ芝生ヲ作ルコトナク常ニ尖圭狀デアルサブロー氏著書ニハ此種ノ扁平ナ芝生ニ就テ記シテナイカラ時トスルト此種ノ尖圭菌ナルカニ就テ疑ヲ生ズルコトガアル

巨大培養ノ場合ニモ著者ノ場合ニテハ（サブロー氏規定ノ標準培養基ヲ用ヰズ）多少外觀上ノ差異ヲ來ス著者ハ三例ニ於テサブロー氏書中ノ寫眞ト全ク同一ナ外觀ヲ呈スルモノヲ得タ卽

狀ノ菌苔ヲ生ジテ其基底ニ赤褐色ヲ呈スル」モノト培養上竝ニ菌學上稍〻近イヤウニ思ハレルノデアル

次ニ著者ノ得タル本種ガ川口恒造氏ガ足部ノ濕疹樣疾患カラ得タ結節性白癬菌（例ヘバ此ノ第二例伊藤某氏種ノ如キ）ト全然一致シナイ事ハ既ニ「汗疱ニ就テ」ノ第二報告ニ論ジタ通リデアル第一氏ノ該菌ハ決シテ尖圭狀形態ヲ表ハサヌ第二ニ葡萄狀芽胞

第三種　紫色白癬菌　Tr. violaceum.

著者ハ二例ノ頭部白癬・一例ノ環狀白癬ヨリ本菌ヲ得タ本菌ハ其特徴ガ著シク且ツ其鑑別ガ困難デナイカラ此ニハ詳細ノ記述ヲ避ケル

臨牀的所見　（甲）頭部表在白癬型

第九例　陳某男十歳奉天讀書人家族大正八年六月五日診

現症　全頭部ニ散在シテ病竈ガアリ一ツ一ツノ大サハ季指頭大デ禿髮面ヲ呈シ或ハ發毛ノ稀薄ヲ來シテ居リ其間ニ病毛ヲ見ル皮膚ノ炎症ハ著シカラズ且ツ是等ノ部分ニ粃糠樣鱗屑ガ附著シテ居ル

第十例　川原某男十四歳奉天在住小學生大正八年十月八日診

（乙）小水疱性疱疹型

第十一例　Ъ·Ю八歳女子露西亞人大正八年十月六日診

現症　右ハ此夏以來當地ホテル滯在中デアツタガ數日前ニ始メテ發生シタ部位ハ右前膊ノ尺骨側デ腕關節ニ近ク其處ニ小水疱ノ連鎖ニヨリテ圈狀疹ヲ作ツタ色ハ淡紅色デ小水疱ノ上竝ニ圈ノ裡ノ皮膚面ニ鱗屑ガ附著スル（插入寫眞圖第十二圖）

鏡檢　第九例第十例ノ毛髮ニ寄生シタモノハ不幸ニシテ鏡檢ノ

及ビ紡錘狀芽胞ノ形成ガ著シ著者ヲ以テ之ヲ觀レバ氏ノ結節菌ハ寧ロ甚ダ石膏樣菌ニ酷似スル但シ氏ノ記載ガ精密デナイカラ確定ニ之ヲ批評シ判斷スルコトガ出來ヌ

動物試驗　動物試驗ハ平見種ニ於テ陽性成績ヲ得タ其狀況ハ「汗疱ニ就テ」ノ第二報告中石毛種ト同型デアルカラ今其記述ヲ略ス

Fig.12 ベルタ種(Tr. viol.)
ニヨル皮膚白癬

機ヲ逸シタ其故此菌ガ確實ニ髮內菌デアルト云フコトハ實證シナカツタ

第九例ノモノハ十月六日ニ鱗屑ヲ鏡檢シテ插入寫眞圖第十三圖ノ如キ要素ヲ見タ第一ハ短大ノ節ヲ有スル菌絲ノ連鎖デ三ミクロン位ノ幅員ガアル或ハ單輪廓或ハ復輪廓ヲ呈シタ略〻直線的ニ走ル尚太クシテ數珠狀ヲナシ途切レ易イモノモアツタ比較的細イ菌絲ハ其幅二ミクロン位デ節ガ甚ダ長ク且彎曲スル內部ニ小サイ顆粒モ見ラレタ總ジテ培養基上ノ形ト一致スル

digitations poilues ガ立派ニ出來ル（附圖第IX表第五圖及ビ插入寫眞圖第十圖1・2・3）

麴寒天上ニモ亦能ク尖圭狀ノ菌苔ヲ作ル

馬鈴薯上ニハ乾燥樣光澤ノ硬固ナル外觀ヲ呈スル純白ノ小點ヲ作リソレガ又融合シテ面ヲナス

變態　サブロー氏ハ本菌ニプレオモルフィズムノ狀態ヲ見ナイト記シタ然シナガラ著者ノ場合ニハ往々斜面培養ノ舊イモノハ白色絨毛狀ノ扁平ナ芝生トナリ其裏面ガ葡萄酒紅トナルモノヲ見タ（插入寫眞圖第十圖4・5）然シナガラ是レトテ之ヲ特ニ移植スルト又尖圭狀隆起ノ菌苔ヲ作ルノデアル

菌學　菌學上ノ所見ハ各菌株皆一樣デアルソレデ岩穴狀菌ニ酷似シテ居ル唯岩穴狀菌ニ在リテハ外芽胞ヲ附著スル枝ニ分岐シテ複雜ナルモノ又葡萄狀ニ簇生シタル外芽胞ガ割合ニ多イガ尖

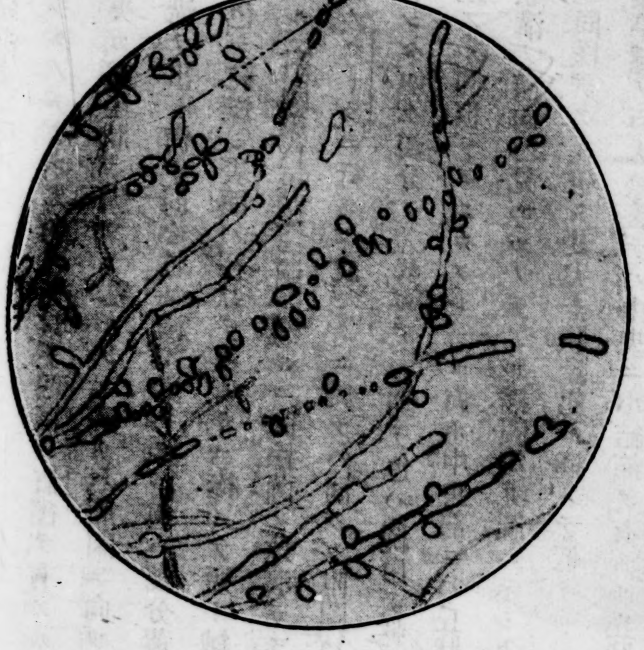


Fig. 11　平見種葡萄糖寒天安置培養法第十日

圭狀菌ニ於テハソンナ複雜ナモノハ少クアッテモ極ルヂマンテエルデ主トシテ單枝ノ兩側ニ一列ニ梨果狀ノ芽胞ヲ附ケテ居ル réserves protoplasmique intra-myceliennes ハ亦此ニ於テモ見ラレテ殆ンド枝ヲ沒シタ迹ニ唯外芽胞ノミガ一列或ハ二列ニ排列シテ居ルノヲ見ル菌絲ノ尖端ハ往々棍棒狀ニ膨脹スル（massues terminales）紡錘狀芽胞（fuseaux）ノ發生ハ之ヲ見ナイガ舊イ培養デハ棍棒狀尖端ガ芽胞トナッテ離レテソウ云フ形ヲナスコトガアル然シ其レガサブロー氏ノ所謂 fuseaux デナイ證據ニハ菌絲ノ側カラ葉狀ニ出ルコトハナク又其分室ガ四五室ノモノモアルガ亦一二室ニ過ギナイモノモアル（「汗疱ニ就テ」第二報告參照）上述ノ如ク著者ガ得タル尖圭狀菌ノ顯微鏡像ハ略〻一樣デアッテ楠氏ノ記述ノ如キ典型ガナイ楠氏ノ黃色塊狀ノ菌苔ヲナスモノハ菌絲側外芽胞ノ形成ナククラミドスポール多ク菌絲太ク且ツ其分芽盛デ稍〻菫色菌ニ似テ居ルガ著者ノ場合ニハサウ云フノヲ見ナカッタ又楠氏ノ所謂「頑癬及ビ爪菌病ノ病原菌トシテ檢出セル白色絨毛狀菌苔ヲ形成スル結節狀菌」ノ如ク「紡錘狀芽胞剩多ニ附著シ其狀豆莢ノ豆蔓ニ垂下セルガ如キ」狀態ヲ呈スルモノヲ見ナカッタ元來サブロー氏ハ尖圭狀菌ニ末端腫脹 renflement massué terminale ヲ認メテ居ル是ハ末端クラミドスポール chlamydospore terminale ニ相違ナイガ石膏樣菌ナドノソレトハ大差ガアル著者モ「汗疱ニ就テ」ノ第二報告ノ尖圭狀菌論ニ於テ著者ガ汗疱樣疾患カラ得タ尖圭菌ニ紡錘狀芽胞ノ外貌ヲ呈スルモノアルコトヲ云フタガ然シ何レノ場合デモ豆蔓ニ豆莢ノ附著スルガ如ク附著スルノヲバ見ナカッタデ著者ノ場合デハ楠氏ノモノト比較スルト其「頭部白癬ノ病原菌トシテ白色絨毛

潤性ノ光澤ヲ有シテ居ル（紫色菌モ時トスルト第二第三代ノ培養デ本菌ノ如キ色澤ヲ取ルコトガアル）千九百十年ノサブロー氏ノ著書ニ據ルト同氏ハ唯一例ノ本種ヲ得テ居ル其レハオデッサカラ來タ猶太系ノ露西亞人ノ小兒ノ頭部白癬カラ得タノデアル此培養ハ紫色菌ヨリハ發育能ク六週間位デ直徑四糎位ニナル

葡萄糖寒天上ノ培養ハ其表面ガ扁平デ中心ニハ稍、大キナ乳頭狀ノ突起ガアルエルレンマイヤ氏マトラノ上ノ巨大培養デハ同ジク乳頭狀（或ハ棘狀）ノ突起デ輪形ガ出來ルガ其中心部ハ却ッテ無棘禿滑glabreデアル交代ヲ重ネテモソウ云フ特徴ハ變ラナイソウ云フ風ニ表面ガ濕潤性デ且ツ略、滑平ダカラサブロー氏ガ其レニ據ッテ命名シタノデアル確カニ紫色菌ノ變種デ鏡檢上・菌學上竝ニ臨牀上紫色菌ト大差ヲ見ナイ又モルモットニ移植スルコトガ出來ナイ

右ハサブロー氏ノ記述ニ據ッテ誌シタノデアルガ著者ハ二例ノ本菌ニ遭遇シタ奇ナルコトニハ孰レモ爪甲白癬デアル始メ著者ハ之ヲ以テ尖圭狀菌ノ一種ダト考ヘタガ後ニ禿滑菌デアルコトヲ知ッタ著者ハ尚ホ培養ニ成功シナカッタ爪甲菌病デ鏡檢上所見本菌ノ爪甲中ニアルモノト殆ド差別ナキモノ二例ニモ遭遇シテ居ルカラ必ズヤ滿洲—又日本（本例ハ孰レモ日本婦人）ニ本菌ノサウ少クナイコトヲ信ズルノデアル

又楠氏ガ「頭部白癬ノ病原菌ニシテ黃色塊狀ノ菌苔ヲ生ズルモノ」ト稱セラレタルハ純マルトーゼ寒天培養基上黃色褐色ヲ呈シ周圍ニ放射狀ニ突起ヲ射出スル星狀菌苔デ培養基中ニ深ク嵌入スル傾向ガアルモノデアル其著色寫眞（本誌第十三卷附圖第七表第二第三圖）ニヨリ又其顯微鏡像（本誌第十四卷第七一三頁第十三圖）ニ據ルニ我禿滑菌ニ似タ所ノアルヲ發見スル唯我本菌ニ於テハ菌學上末端ノ葡萄狀芽胞ガ闕ケテ居ル但シ氏ノ記述ノミニ據リテ本菌トノ異同ヲ考フルハ困難デアル

臨牀的所見

第十二例　井川某女四十七歲長崎縣人奉天在住・診斷・爪甲白癬大正八年六月四日診

現症　右側ハ凡テノ指ノ爪甲ニ於テ左手ハ第一第二指ノ爪甲ニ於テ罹患シ表面ノ不平・粗鬆・龜裂・鱗屑狀剝離ヲ來ス余ノ初診（五月二十六日）以前ニハ尙手背及ビ足背ニモ表在性白癬（?）ガ存シテ居タサウデアル（第十五圖參照）

第十三例　金原某女三十三歲長崎縣人奉天在住・診斷・爪甲白癬兼手掌ニ於ケル汗疱樣濕疹大正八年十一月二十五日診

既往症及現症　昨年來兩手掌ニ小水疱性ノ發疹ヲ生ジ殊ニ夏期ニ於テ增惡スル現在ハ兩手掌ハ乾燥シテ小ナル鱗屑ノ形成ヲ見

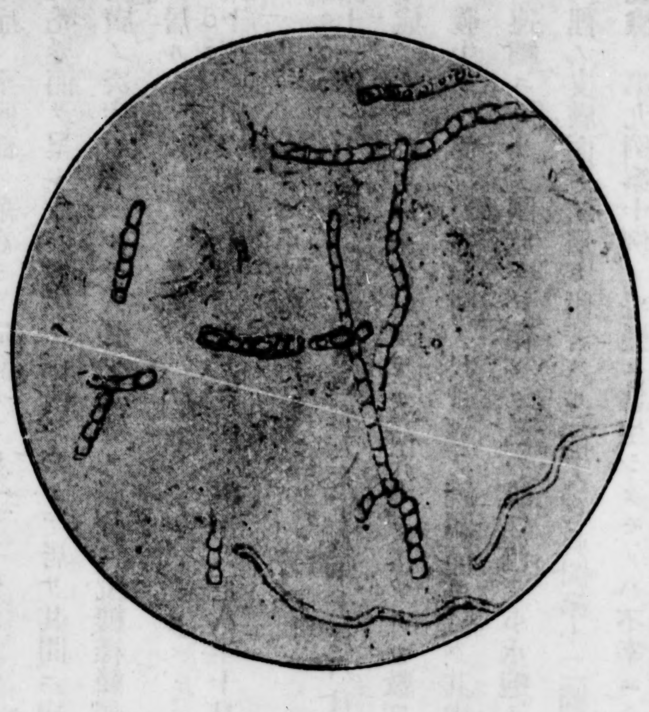


Fig.13 鱗屑中ニ於ケルベルタ種

培養　マルトーズ又ハ葡萄糖寒天斜面ニハ移植後六日頃カラ灰黄色ノ小點ガ現ハレ其レガ增大シテ二週間許リ經ツト紫色トナル大サハ小ナルハ鋲頭大デ環狀ヲ爲ス傾向ガアル大ナルハ爪甲大デ衣皺樣ノ褶襞ヲ生ズルソレハ幾分放射狀ヲナス表面ハ濕潤樣ノ光澤ヲ呈スル(附圖第IX表第六圖)

三%ペプトン寒天ノ上デハ發育ガ一層惡イソシテ代ヲ重ヌルト紫色ガ微褐色ニ褪セルコトガアル麴寒天ノ上デハ割合ニ良ク發育シ其形ハ糖ノモノト大差ガナイ孰レニシテモ發育ハ餘リ良クナイガ糖寒天ノマトラノ培養デモ直徑ガ一糎强ニシカナラナカツタ

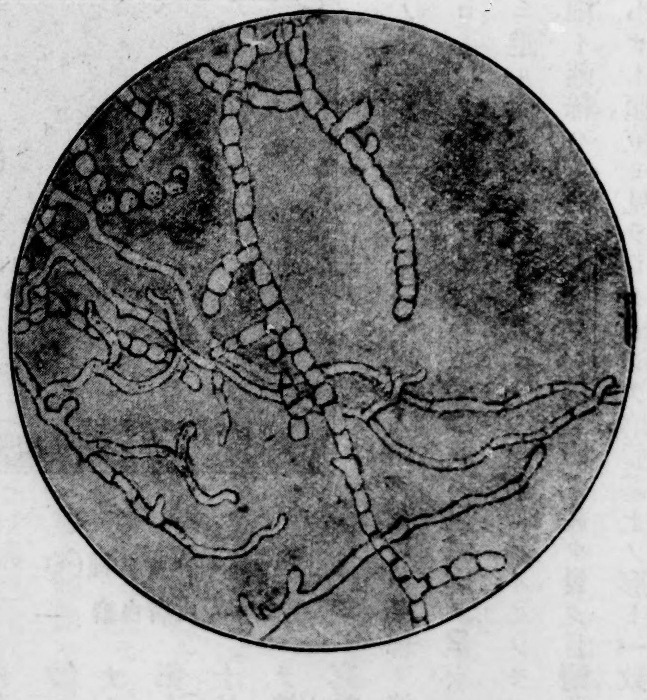


Fig.14 陳種葡萄糖斜面第十四日培養

馬鈴薯ノ上デハ著者ノ場合ニハ純白色ノ點狀ノ聚落ヲ作ツタ外觀ガ堅ク且乾燥性デ表面ハ粉狀デアツタガ所ニヨリテ短絨毛狀デアツタ

菌學　安置法デハ發育ガ惡イ陳氏種ノ二週間ノ斜面培養ヲ取ツテ見ルト第一ニ稍、細ク且幾分屈曲性ノ菌枝ガアツテ節ハ割合ニ短イソレカラ側枝ノ出ルコトガ多イ菌絲ノ先端ハ膨脹ヲ示サナイ菌絲ノ内部ニハ顆粒ガ藏セラレテ居ル次ニ稍、太ク且ツ短ク數珠狀ニ連接スル菌絲ガアルソレカラモ側枝ガ多ク出ル此方ハ寧ロ直線的ニ走ル(挿入圖第十四圖)

動物試驗　陰性

第四種　禿滑狀白癬菌 Tr. glabrum Sabouraud.

本菌ハ紫色菌ノ變種デアル是ハ其色ガ(全體ニハ勿論部分的ニモ)決シテ紫ニナラヌ却ツテ淡褐色デアル其表面ハ濕

作ル

先ヅ爪甲ヲ移植シテ後約一週間ニシテ灰黃色ノ小點ヲ生ジ其表面ハ濕潤性ノ光澤ヲ有シテ居ル培養ハ深ク且緊密ニ培養基中ニ入リ針ヲ以テ離スコトガ困難デアル

加糖寒天斜面デハ中央ガ圓キ小結節ヲ作ツテ隆起シソレヨリ周圍ニ緩カニ下ル其際微ニ少數ノ放射溝ヲ作ルコトガアル又或場合ニハ極細カイ縮緬樣又ハ棘樣ノ皺襞又ハ突起ヲ作ルコトガアル（附圖第IX表第八圖及第十圖）色ハ濕潤性光澤ヲ有スル帶黃淡褐色デアル

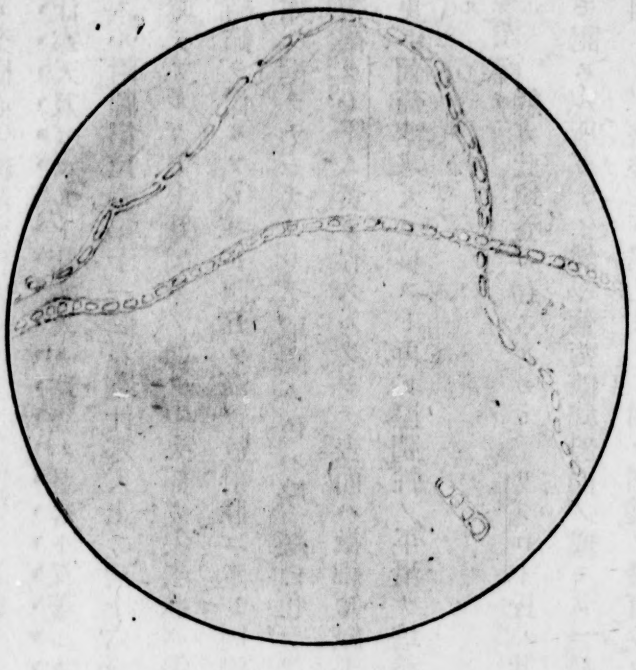


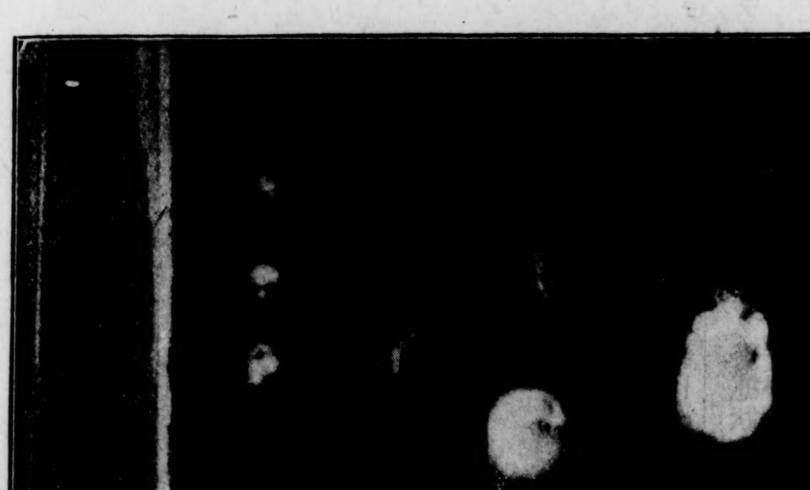
Fig.18 金原種(Tr. glabrum.)ノ爪甲中ニ於ケル形態(25.XI.1919)

麹汁寒天ノ上ノ培養ハ葡萄糖又ハペプトン寒天上ノモノト著シク異ルソレハ細イ純白色短絨毛デ半球上ニ隆起スル（第十九圖e）

三%ペプトン寒天デハ發育ガ稍〻惡ルク低イ圓板ヲツクリ少數ノ皺襞ガアリ中央ニハ必ズ小結節ヲ作ル表面ハ滑平デ濕潤性ノ臘樣光澤ヲ有シ其色ハ褐色味ヲ帶ビタクレエム色デアル（第十九圖c）

馬鈴薯ノ上ニハ雪白ノ小點ヲ作リ光澤ハ乾燥狀デ且ツ硬ク見エル寒天上ニ於ケルガ如キ濕潤性ヲ失フ

エルレンマイヤー氏マトラニ施シタ巨大培養デハ或時ハ附圖IX表第九圖ニ示シタヤウナ外觀ヲ呈スルコトガアル是レハ葡萄糖寒天デ六十一日ノモノデアルガ中央ニ羽二重樣ノ總ヲ作リソレカラ稍〻深イ溝ガ放射狀ニ走リ其邊緣ハ遞降的ニ深ク培養基中



a b c d e

Fig.19 a. Tr. violaceum（陳文治葡萄糖寒天移植後第六日）
b. Tr. glabrum（金原種同同）
c. „（同ペプトン寒天第三十一日）
d. „（井川種葡萄種寒天第二十二日）
e. „（井川種麹汁寒天第十九日）

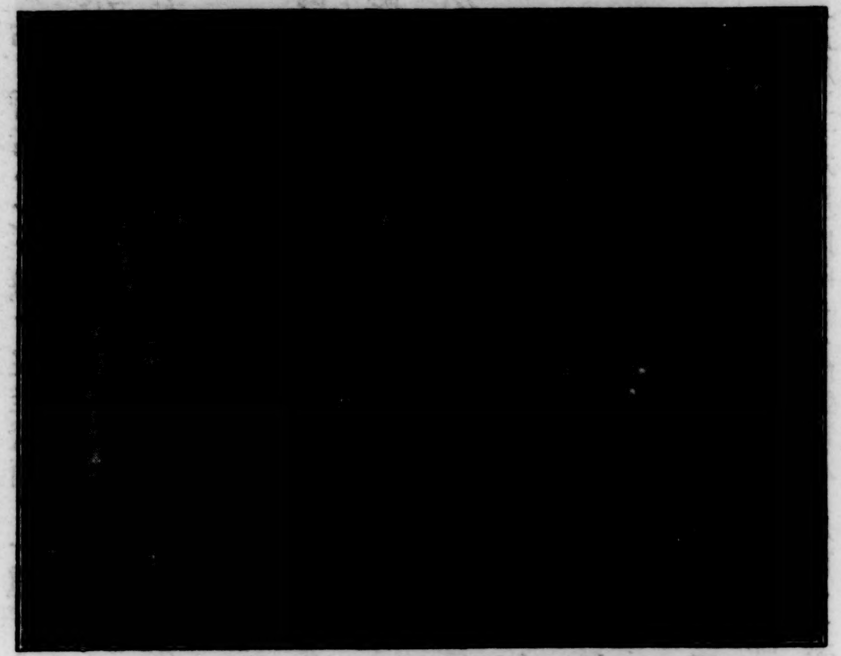


Fig.15 中川某女ノ爪甲炎

Fig.16 金原某女ノ爪甲炎

既ニ水疱ハ之ヲ見ナイ鱗屑ハ指間ニ著シイ爪甲ノ方ハ右第二指ヲ除ク外ハ雙手ノ凡テノ爪甲ガ多少ノ程度ノ差コソアレ皆侵サレテ居ル（挿入寫眞圖第十六圖）

鏡檢　第十二例ニ於テハ爪甲種ノ菌絲ハ太クシテ短キ節ノ連鎖ヲナシ其各細胞ハ顯著ナ二重輪廓像ヲ呈スル此種ノモノハ其幅ガ四・五ミクロン位デ一細胞ノ長サハ七ミクロン前後デアル別ニ同ジ型デ其幅前者ノ二分一位ノモノモアル又既ニソレガ一箇ノ楕圓形又ハ圓形芽胞トシテ分離シタノモアル（第十七圖）次ノ例モ略ヾ同樣デ菌絲ノ幅ハ約三ミクロンデアル（第十八圖）

培養　本菌ハマルトーズ寒天・葡萄糖寒天・麴寒天・三％ペプトン寒天等ノ斜面又ハマトラニ於テ殆ド一樣ノ外貌ヲ有スル培養ヲ

Fig.17 井川種(Tr. glabrum.)ノ爪甲中ニ於ケル形態(4.VI.1919)

ツテ太クシテ不規則ナル形トナリ且ツ短ク分節シ遂ニソレガオオスポラ樣ノ連鎖トナル

カク本菌ハ菌絲・クラミドスポール・芽胞連鎖ノ三種ノ要素ヲ以テ常型トスルガ著者ノ場合外芽胞ハ兎ニ角發見セラレタルハ奇トスベキデアル是等ハ麴汁寒天ニ培養シテ白色短絨毛狀ノ尖主狀菌ノ如キ外觀ヲ呈シタ(第十九圖e)モノニ於テノミ唯甚ダ少數ニ於テ見ラレタノデアルソシテソレヨリ遊離シタル芽胞ハ西洋梨ノ形狀ヲ呈スル(第二十一圖a)但シ此培養ハ(約四十日ノモノニ就テ見ル)主トシテ長キ菌絲ヨリ成ルモノデアッタ但シ是ニ由ツテ觀ルニ麴汁寒天ガ本菌發育ニハ從來培養セラレタル他ノ基ヨリモ遙ニ好適ナルコトヲ知ラレルノデアル

次ニ葡萄糖寒天上ニ於ケル白色短絨毛狀ノ培養(變態?(プレオモルフイズム)第十九圖d培養下部ノ聚落)ハ菌學上主トシテ不規則ナ芽胞連鎖(第二十一圖b)ヨリ成リ其間僅カニ菌絲ヲ混ジタ

(注意)サブロー氏モ千八百九十三年中菫色菌ノ白絨毛性ノモノヲ得而シテソレニ側生外芽胞ヲ見出シタコトヲ記シテ居ル(Les Teignes, P. 647.)

動物試驗 數回反復シテ試ミタガ常ニ陰性デアッタ

第五種 星芒狀石膏樣白癬菌 Tr. gypseum asteroides Sabouraud.

本菌種ニ關シテハ曩ニ「汗疱ニ就テ」ノ第二報告ニ於テ詳論シタカラ此ニハ唯簡單ニ症例ヲ記シ且爾餘ノ項ニ就テ少シノ注意ヲ附加スルニ止メル

臨牀的所見 (甲)頭部白癬型

第十四例 中原伊一郎十歲京都府生・鈴嶺在住・診斷頭部表在白癬兼禿瘡大正八年二月二十四日診

第十五例 中原經次郎五歲(右ノ弟)診斷頭部表在白癬同日診

第十六例 安田久卿九歲福島縣人鈴嶺在住・診斷頭部表在白癬兼禿瘡同日診

右三人ハ孰レモ鈴嶺ニ在住シ經次郎以外ハ其地ノ小學校ニ通學スルモノデアル而シテ附添者ノ言ニ據レバ當時同地ニ於テハ本型ノ白癬流行シツヽアツタトノ事デアル孰レモ約半箇月前カラ罹病シテ第十四例ノ場合ヲ除ク外ハ孰レモ數多ノ膿瘍ト局所淋巴腺腫トヲ伴ツテ居ツタ表在性ノモノハ其病竈拇指頭大デアツタ毛髮ハ切斷シ居リ且ツ厚イ白痂及ビ脂漏性濕疹ヲ兼有シテ居ル

第十七例 柳澤清十歲橋頭在住・診斷頭部表在白癬兼顏面斷髮疱疹大正八年三月二十一日診

(乙)無毛部斑狀白癬型

第十八例 柳澤靜江六歲(右ノ妹)診斷顏面斑狀斷髮疱疹同日診

當時橋頭ニ於テモ同樣ノエピデミーガアリ兄ノ方ハ一ケ月以前ヨリ頭部ニ生ジタ頭部ニ於テハ各病竈ハ相融合シカナリ廣イ面ニ於テ厚イ白色痂皮ト脂漏性濕疹ヲ來シ毛髮ハ所々其爲メニ相膠著シテ居ルガ斷髮脫毛ハ著シクナイ

第十九例 大橋某男七歲長崎縣人奉天在住・診斷 右頸部小水疱

ニ埋沒スル色ハ帶褐クレーム黃デアル然ルニ或場合ニハソレトハ稍、異ツタ形ヲ取ル附圖第十圖ハマルトーズ寒天上ノ五十日目ノ培養デアルガ放射溝ノ發達ガ著シクナリ表面ハ略、同心性ノ圏ヲ畫イテ細小ノ乳頭狀突起ガアル色相・光澤ハ全ク同ジデアル又同心ニ結節ヲ作ル

麴汁寒天及ビマルト汁寒天ノハ培養ハ是等ト又著シク其形體ヲ異ニスル附圖第IX表第十一圖ハ麴汁寒天上ノ三十六日目ノ培養ノ寫眞デアルガ尖圭狀菌カノ如ク中央部ガ尖圭狀ニ尖ツテ中心ニ小結節ヲ作リソレヨリ四五ノ溝ガ放射狀ニ走リ周邊ハ帽子ノ緣ノ如ク裾ヲナシテソレヲ取圍ム色ハ略、純白色デアルガ部分的ニ帶褐クレーム黃ガ存スルソシテ表面ハ微細短絨毛狀デアル之ヲ更ニ葡萄糖寒天ニ移スト再ビ濕潤性ノ平滑ナ培養トナルノデアル

變態（プレオモルフィズム）ガ此培養ニ存スルカ? サブロー氏ハ其事ニ就テ毫シモ記スル所ガナイ然シ葡萄糖樣斜面ノ或モノニ於テ插圖第十九圖ノdノ培養ノ下部ノモノ、如ク褶襞ノ發育少クシテ廣キ滑平面ヲナシ其色ハ寧ロ灰白色トナツテ且ツ極微ノ白絨毛ヲ生ジタノハ或ハ變態ノモノデアラウト且ツ麴汁寒天上及ビマルツ浸出液寒天ノ特殊ノ形貌ハ同基上ニノミ特殊ノモノデアルカラ之ヲモツテ變態ト見做スコトハ出來マイ

菌學 安置培養法デハ發育ガ割合ニ緩慢デ一ケ月經ツテモ尚ホ長イ菌絲ヲ生ズルノミデアル其際一箇ノ外芽胞ヲモ見ナイガ又クラミドスポールヲモ發見セヌノデアル

Fig.20 井川種(Tr. glabrum.)
第四代葡萄糖培養第二十日

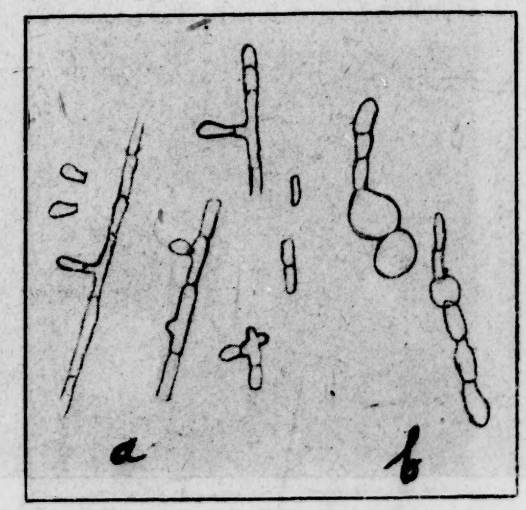


Fig.21 白絨毛狀ヲ呈スル金原種
(Tr. glabrum.)
a）麴汁寒天斜面第四十日培養ヨリ
b）葡萄糖寒天斜面第四十一日培養ヨリ

ソレデ斜面寒天ノ培養カラ小片ヲ採ツテ之ヲ鏡檢スルト菌絲ハ兩緣平行ニ走リ或ハ極緩徐ナ膨脹及緊縮部ガアツテ中ニ小顆粒ヲ藏シ所々ニクラミドスポールヲ形成シテ居ル菌絲ハ割合ニ繁テ分枝ヲ出ス傾向ガアリ又穗狀芽胞樣ノ短イ突起トナル（les bourgeons latéraux 插入圖第二十圖）然シ又實際ソレガ外芽胞デアルコトガアル（同第二十一圖a）菌絲ハ漸ク其纖維樣形狀ヲ失

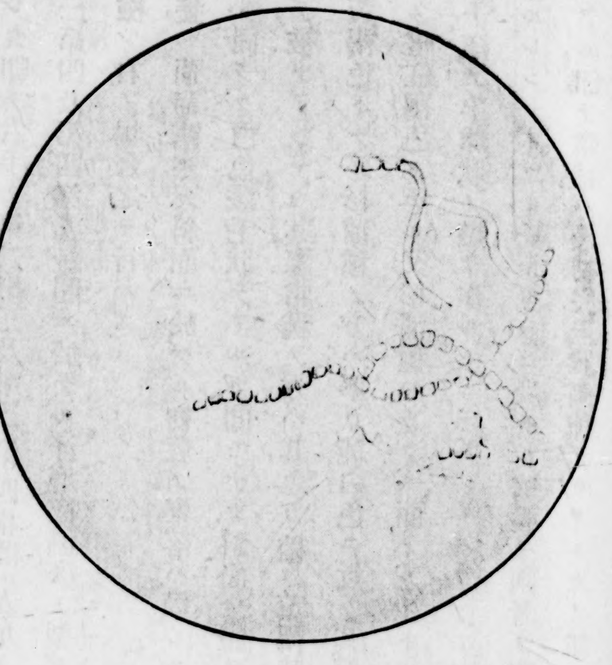


Fig.25 大橋種皮膚鱗屑中ニアル形狀

Fig.26 大橋種
麴汁寒天第十三日

陷沒トナルコトモアル）光芒ノ尖端ハ相集ツテ略〻圓形ヲナス色ハ始メハ純白色デアルガ後ニハ帶黃褐調ノ白色トナル（附圖第IX表第十二圖ハ葡萄糖寒天基上ノ柳澤種同第十三圖及插入寫眞圖第二十六圖ハ麴汁寒天基上ノ大橋種デアル）

初代ノモノモ培養ガ舊クナレバ又代ヲ重ネテ行ケバ漸次變態ヲ來シ遂ニ圓形ノ白羅紗樣表面トナル其際多少黃色調ヲ帶ビ來ル

馬鈴薯上ニハ白色粉狀扁平ノ點又ハ平面ヲ作リ所々ニ短絨毛ヲ現ハス

菌學　安置培養法ニ於テ其所見殊ニ顯著デアツタ大橋種ニ就テ見ルニ四％葡萄糖寒天ヲ用ヰ三日ニシテ分枝著シクナリ翌日ハ分枝ニ又更ニ小サキ枝ヲ出シ六日ニシテ大ナル葡萄狀芽胞ガ多數ニ發生シタ其他ニ尚ホ長キ莖ヲ有スル單純性ノ外芽胞莖ヲ見ラレタ尚ホ數日ニシテ多數ニシテ而モ廻轉數甚ダ多キ卷鬚狀器官ガ現ハレタ後尚ホ數日ニシテ見ルト紡錘狀芽胞ノ外ニ長キ菌絲ノ間ノクラミドスポールガ數箇又ハ數十箇連珠狀ニ聯結シテ居ルモノモ見ラレタ又菌絲ノ細胞ガ其一端ラケツト狀ヲナシテ他ノ細胞ト接スルモノモアツタ

動物試驗　陽性・髮外菌

鑑別　本種ガ石膏樣菌デアルコトハ最早疑ヲ容レザル所デ唯問題トナルハ其 Asteroides ナルカ Radiolatum ナルカノ點デアルサブロー氏ノ標準培養基ヲ有セザル著者ハ培養ノ外形ニ由ツテ之ヲ判別スルコトハ困難デアル若シ著者ノ用ヰタル培養基デモ略〻サ氏記載ノ區別ガ尚ホ存スルトシタナラバ Radiolatum ノ方ハ其色ニ薔薇色々調ガアツテ且ツ光芒ガ混亂シテ居ル著者ノ場合ハ色ハ幾分黃色調ヲ帶ビルガ紅味ハナイ且ツ光芒ガ割合ニ纖細デアル（但シ大橋種ハ幾分混亂シテ居ル）

次ニ菌學上ニ兩者ヲ區別スルコトハ困難デアルサ氏ハ一菌絲細胞端ノラケツト狀ニナルコト及ビ培養ノ中心部ニ毛狀ヲナス菌絲ヲ見ルコトヲ後者ニ認メテ居ル著者ノ場合モ大橋種ニハラケツト狀菌絲ヲバ見ル

然シ要スルニ兩石膏樣菌ハ甚ダ近キ關係ニ於テ存スルモノデ強ヒテ分類スルニモ當ラヌモノデハアルマイカト思フ著者ハ上述ノ凡テヲ Asteroides トシテ茲ニ總括スル

Fig.22 左ヨリ中原經二郎安田久次郎中原伊一郎
(Tr. asteroides. ニ由ル頭部白癬)

性斑狀白癬大正九年三月六日診

現症 右側頸部ニ二錢銅貨大ノ圓形紅斑アリ中央部ハ色薄ク邊緣部ニハ少數ノ小水疱アリ一般ニ落屑ガ著シイ

鏡檢 (甲)頭部白癬・中原及安田ノ毛髮ニ寄生セルモノヲ見ルト髮外菌デ直徑三ミクロンノ芽胞ガ多數毛髮外表ニ密集シテ居リ毛髮内部ニハ菌絲ヲ見ナイ

又柳澤ノ方デハ三ミクロン大ノ芽胞毛外ニアルコトハ上ノ如クデアルガ又髮ノ内部ニモ短キ節ヲ有スル菌絲ガ珠數狀ノ連鎖ヲナシテ毛髮ノ長軸ニ平行シテ走ル

(乙)無毛部斑狀白癬デハ大橋ノモノヲ見ルト約三ミクロンノ幅員ヲ有スル菌絲ガ彎曲シテ走リ其一部ハ顆粒ナク節甚ダ長ケレドモ多クハ甚ダ短節デ方形又ハ長方形ノモノ、連鎖狀ニ聯結スルヲ見ルノデアル(插圖第二十五圖)

Fig.23 柳澤靜江(同小水疱性白癬)

Fig.24 大橋某男(同)

培養 始メハ白色絨毛狀ノ聚落ヲ作リ發育スルニ從ツテ漸次光芒狀トナル斜面寒天デハ其光芒ハ直キ混雜シテ早ク白羅紗狀ノ平面ト化スガ巨大培養ニ於テハ纖細ナル光芒ガ極メテ美シク生ズル卽チ中心ニ低キ圓形ノ結節ヲ作リ(結節生ゼズシテ不規則ノ

七日目ニハ盛ニ葡萄狀芽胞ヲ發生シ又單純ナ穗狀芽胞ヲ附タル長キ枝ガ見ラレタ此狀態ガシバラク續キヤガテ菌絲內容ノ吸收ガ現ハレル此際紐狀ニ竝列シタ芽胞ハ梨狀又ハ橢圓形圓形等デ大小ニ相違ガアル但シ常種ノ方ニハ分枝狀葡萄狀芽胞 grappes ramifiées ガ多イガ杉浦種ノ方デハ其葡萄狀芽胞ハ主ナ枝マタハ僅デ且ツ短イ側枝ニ簇生シタモノガ多イカウ云フ點カラモ常種ハラヂアンス種ニ近ク杉浦種ハ寧ロデンチクラーツム種ニ近イ無論紡錘狀芽胞・卷鬚・結節狀器官等ノ發生ヲ見ナイ

動物試驗 嚮キニ著者ガ得タル雪狀菌ニテハ動物試驗ニ成功シナカッタガ今回ハ兩種共ニモルモットニ移植スルコトガ出來ソレハ純粹ノ髮外寄生デ小型ノ芽胞及ビ細キ菌絲ヨリ成ル外套ヲ形成シタ髮內ニハ一ツノ菌絲モ認メラレナカッタ

第七種　白色黃癬樣白癬菌 Tr. faviforme album Sabouraud.

本種ハ最近一例得ラレタモノデアル病毛ノ顯微鏡像ハ髮內外性デアルガ培養ノ外觀及ビ菌學的所見ニ依ッテ黃癬樣菌ダト見做スニ至ッタ

本種ハ夙ニ F. Bodin 氏ニ據リテ研究セラレ元來馬・驢馬・牛等ノ病原菌デアル同氏ノ得タルモノハ褐色及ビ灰白色ノ培養ヲ造ルモノデ氏ハ之ニ Tr. verrucosum ノ名稱ヲ與ヘタ著者ノ場合ハボダン氏ノ此名稱ヲ以テ最モ適當ノモノト考ヘラレル

サブロー氏ハ本種ヲ三別シテ Tr. faviforme album, discoides et ochraceum トナシタ此分類法ニヨレバ著者ノモノハ白色種ニ屬スルモノデアラウ白色種ハ同氏ハ唯一例ニ於テ得ラレ而モソレハ無毛部白癬デアッタ

臨牀的所見　第二十二例　李聚仲十五歳奉天人男生徒・診斷頭部表在性白癬大正九年六月十五日診

此患者ハ本年三月來兩頸部ニ於ケル結核性淋巴腺炎ノ故ニ治療中デアッタガ六月ニ至リ始メテ頭部ノ白癬ニ氣付イタ此疾患ノ既往症ハ明カデナイガ現在ハ主トシテ右側顱頂部ニ二錢銅貨大ノ無毛部ガアッテ他部ノ較ヾ長イ毛髮ト明ニ差別セラレル其部ハ殆ド無毛デ發育ノ惡イ病毛ナドハ見ズ但シカナリ强ク灰白色ノ鱗屑ヲ附著シテ居ル然シ患部ノ周緣ニ在ル發育良キ毛髮ニ就テ鏡檢上容易ニ菌要素ヲ發見スルコトガ出來ル

病毛ハ上述ノ如ク中途ニ切斷セラル、コトナク普通ノ發育ヲナシ根部及ビ頭部皮膚ヲ超エテ出ヅル毛幹部マデモ薄イ灰白色ノ鱗屑デ全部或ハ部分的ニ圍繞セラレテ居ル然シ乍ラ此外膜ハ甚ダ薄ク內部ノ毛幹ヲ透見セシメ且ツ容易ク毛髮カラ離レル然シ顯微鏡的ニハ尙ホ芽胞及ビ菌絲等ノ菌要素ヲ見ルコトガ出來ルノデアル

鏡檢　元來黃癬樣菌ハ髮外菌デアル然ルニ著者ノ場合ニアッテ

第六種　雪狀白癬菌 Tr. niveum.

此種ハ唯汗疱樣ノ足蹠間白癬ヨリ得ラレタ既ニ小稿「汗疱ニ就テ」ノ第二報告ニ於テ此種ノコトヲ記載シタガ此ニ尙二例ヲ追加スル當時ハ動物試驗不成功デアツタカラ幾分疑念ヲ存シテ居タガ今回ハ陽性デアツタソレデ都合四例ノ雪狀菌ガ汗疱カラ得ラレタノデアル

臨牀的所見　第二十例　常炳泰三十六歲奉天人學堂小使大正九年五月十二日診

右ハ約一ヶ月來左足蹠殊ニ第一趾第一第二趾骨內側部同蹠骨內側部及ビ第五趾蹠骨外側部ニ典型的ノ小水疱ノ群生ヲ來シタモノデアル但シ足趾間ニハ病竈ヲ見ナイ

第二十一例　杉浦某男二十二歲靜岡縣人奉天在住學生大正九年五月二十二日診

本患者ハ昨年十二月中ニモ同樣ノ疾患ヲ生ジ今回ト同ジ培養ヲ得タコトガアル今年ハ二月頃ニ發生シ昨年ト殆ド同一部位ガ侵サレタ卽チ右手ノ第二第三及第三第四指間・左足ノ第一第二・第三第四及第四第五趾間ニ於ケル汗疱樣濕疹ノ型デアル

鏡檢　右ノ場合之ヲ行ハズ

培養　葡萄糖寒天斜面ニ於ケル初發ノ聚落ハ孰レモ石膏樣菌ノ時ト同ジク白色絨毛狀デアルガ間モナク斜面全體ヲ絨毛狀ノ芝生デ被ツテシマウ其際常種ノ方ハ其色ガ稍〻黃褐味ヲ帶ビ背面ハ黃褐色トナル杉浦種ノ方ハ寧ロ純白色ニ近ク其裏面ハ葡萄酒樣ノ暗紅褐色トナル又杉浦種ハカク一面ノ芝生トナル前ニ白粉刷子樣ノ半球狀ノ叢ヲ作ル

エルレンマイヤー氏マトラニ於ケル培養モ兩者ノ間ニ多少ノ差ガアル卽チ常種ハ最初尖圭狀菌種デモアルカノ如ク皺襞ヲ有スル短絨毛狀ノ結節ヲ作リ後ニ其周圍ニ平面ノ芝生ヲ生ジ其緣ハ纖細ナル光芒ヲナスノデアル卽チ此形カラシテ常種ノ方ハ Trichophyton niveum radians Sabouraud カ或ハソレニ近キ種類ダト考ヘラレル（附圖第IX表第十四圖）其色ハ培養ガ舊クナルト幾分黃褐色調ガ深クナルノデアル

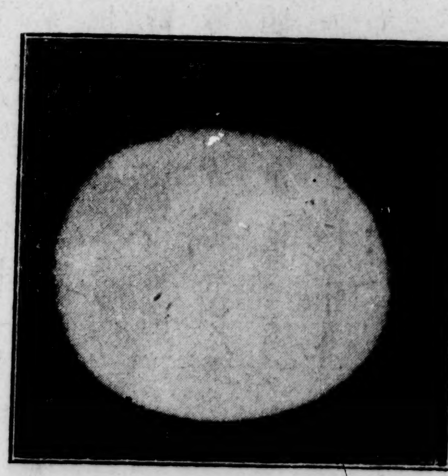


Fig.27 杉浦種麴汁寒天
第二十三日培養

杉浦種ノ方ハ始メハヤハリ白色ノ絨毛狀ノ結節トナツテ隆起スルソレガ段々其半徑ヲ增スニツレテ三四條ノ淺ク廣イ放射狀溝ヲ作ルガ其邊緣ニハ星芒狀ノアレオールガ現ハレナイ略〻圓周狀ヲナスノデアル其色ハ幾分黃褐味ヲ有スルガ常種程著シクナイ（插圖第二十七圖）

馬鈴薯ノ上デハ兩種共ニ純白長絨毛性デ半球上ニ高マツタ白粉刷子樣ノ柔イ培養ヲ作ル

菌學　兩種共ニ其菌學的所見ハ殆ド同樣デアル安置培養法デハ移植ノ翌日ニハ眞直ノ菌絲ヲ出シソレヨリ略〻直角ニ枝ヲ出ス

之レヨリ他ノ斜面ニ移植スルト二十日後ニハ中央ニ皺襞アル半球狀ノ結節ヲナシ其周圍ニ亦狭キアレオールヲ出ス其色ハ微キ黄褐色デ其表面ハ微細粉狀デアル

三％ペプトン寒天斜面ニ於ケル第三代ノ培養ハ其色白色トナリ圓形ノ結節トナッタ

巨大培養ニ於テハ四％葡萄糖寒天及ビ三％ペプトン寒天ニ於テ略々同樣ノ結節ヲ作ル卽チ第二代移植後第十二日ノ所見ニ於テハペプトン寒天上ニハ直徑約二・五糎ノ圓形ノ結節ヲツクリ其中心ガ同心圓性ノ襞ヲツクリソレヨリ不規則ナル放射狀ノ摺襞ヲナス此周縁ハ極メテ微細ナルアレオールヲ作ル表面ハ亦微細粉狀デ色ハ臘樣黄デアル

葡萄糖寒天ノ場合ニ殆ド右ト同樣デアルガ此方ガ結節部トアレオールトノ堺ガ明瞭デアル

麴汁寒天ノ上ニ於テハ其色ガ更ニ紅褐味ヲ帶ビ邊周ノアレオールノ幅ガ甚ダ廣ク結節部二・〇糎・アレオールノ幅一・五糎ヲ算スル

マルト汁寒天デハ結節ノ發育悪シク殆ド移植塊ノ大サニ止マリ且ツ甚ダ發育悪クシテ微細ノ網ヲナス所ノ幅廣キアレオールヲ出ス

馬鈴薯上ニハ黄褐灰色デ臘樣光澤ヲ有スル點又ハ條ヲ作リ處々ニ純白色ニシテ微細絨毛性ノ結節ヲ島嶼狀ニ形成スル（馬鈴薯上ノ培養ノ外觀ハ全クボダン氏ノ記述ト一致スル）

菌學　安置培養法デハ既ニ翌日ニ菌絲ヲ出スガ其尖端ハ多ク chandelier favique ニ類スル分枝狀膨脹ヲ示ス（插入圖第三十圖 a）後一週間許經ッテ見ルト透明ナル菌絲ハ極メテ短ク圓形叉ハ橢圓形ヲナス所ノ節ノ連鎖トナッテシマッテ居ル是等圓形細胞ハ又其側面カラ同ジ形ノ細胞連鎖ノ枝ヲ出ス（同b）或菌絲デハ然シラケット樣膨脹トグラミドスポールトヲ形成スル（同c）又培養ノ初期ニ於テハスポランギウム及スボランギウム嚢ヲ作ル（同d）或菌絲ニアリテハ其側面ニ外芽胞ラシキ突起ヲ出スノヲモ認メル（同e）而シテ培養ガ稍々舊クナルト殆ド凡テ菌絲内クラミドスポールノ連鎖トナルノデアル其際細胞壁ハ顯著ナル二重輪廓像ヲ呈シ内部ニハ大小ノ顆粒ヲ藏スルソシテ繁ク分枝ヲ出スノ傾向ガアル（同f）

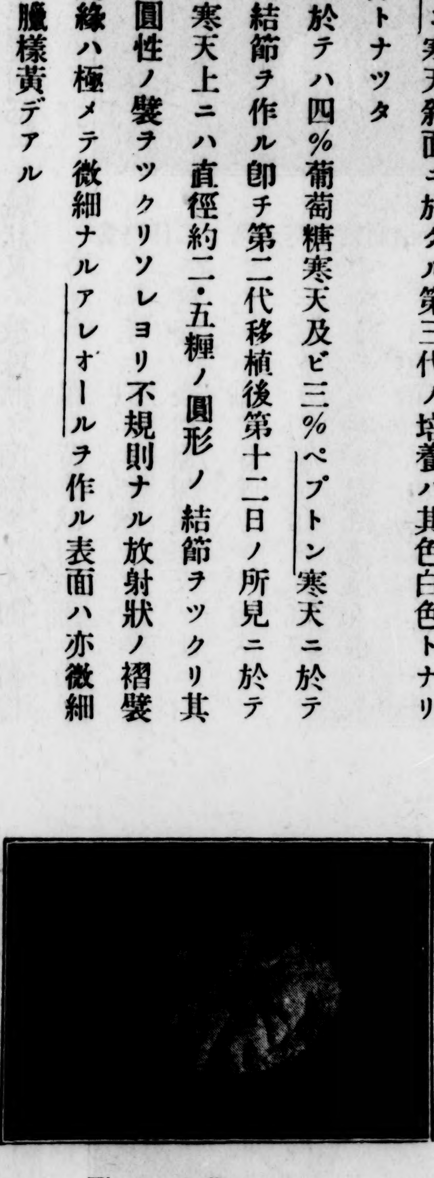


Fig.31 李菌ペプトン寒天第十二日培養

斯クノ如ク菌學上ノ所見モ黄癬菌ニ似タ所ガ多イ

動物試驗　ハ一週間後ニハ移植部ノ鱗屑形成ガ著シクソコニハ幅廣キ菌絲ト大キナ芽胞ガ密集シタ然モ毛髪ノ侵サレタモノハマダ發見セラレナカッタ著者ハソレ以上追究スル時日ヲ有シナカッタ

第八種　衣襞狀白癬菌 Tr. plicatile Sabouraud (?)

著者ノ得タル本種ハ亦其鑑別甚ダ困難デアッタ唯其大芽胞菌ニシテ而モ髪内外菌ナルコト、培養ノ外觀トニ由ッテ

Fig.28 Tr. album.ニ侵サレタル病毛ヲ示ス

ハ常ニ髮內外菌ノ狀況ヲ呈シタ(然シボダン氏ハ其 Tr.verrucosum ハ人間ニ於テハ髮內外性デアルト云ッタ又サブロー氏モ Tr. ochraceum 或ハ Tr. discoides ニ於テ髮內ノ菌絲ヲ認メテ居ル)

髮內ニ於テハ約三ミクロンノ太サノ透明ナ菌絲ガ多數毛髮ト略〻平行ニ走ルノ他尚ホソレヨリ稍〻太ク而シテ極メテ短節ヨリ成ル連珠狀菌絲ガアルソレハ處ニヨリテハ連鎖トナラズニ不規則ナ石垣狀ノ群團ヲナスコトガアル是等ノ圓形又ハ多角形ノ節或ハソレヨリ分離シテ確ニ芽胞ト思ハル、モノハ其直徑七ミクロン內外ヲ有スル

又或ル病毛ニ於テハ三ミクロン以下ノ直徑ヲ有スル芽胞ガ小芽胞菌ノ如ク毛髮ヲ圍繞スル有樣ガ見ラレタ

培養 葡萄糖斜面ノ上ノ初發ノ聚落ハ其發育極メテ緩慢デアリ移植シタル毛髮ノ上ニ十數日ヲ經テ始メテ數箇ノ小結節ヲ作リソレガ中々大キクナラヌソノウチニ周圍ニ放射狀ノアレオールヲ作ル主トシテ其レハ基ノ內部ニ擴マル

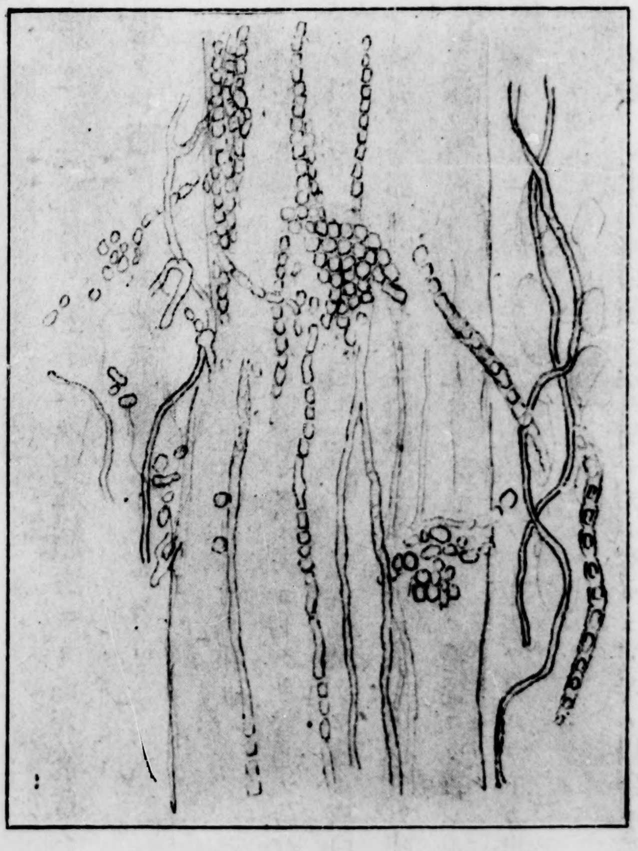


Fig.29 李菌ニ侵サレタル病毛ノ鏡檢像

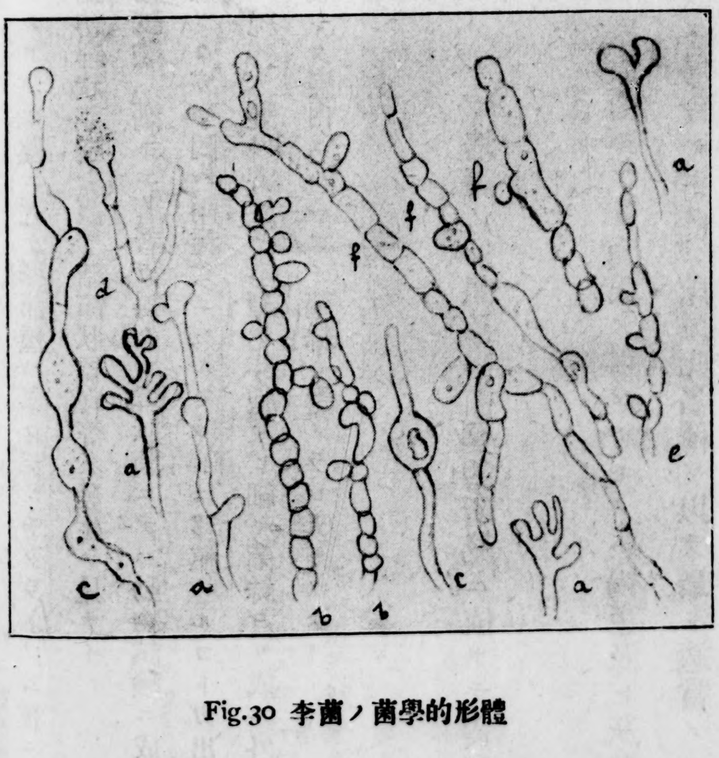


Fig.30 李菌ノ菌學的形體

Fig.35 麹汁寒天斜面第二十七日

徑約一ヶ月ノ培養ニ於テ三糎位デアッテ中央部ガ岩穴狀菌樣ニ陷沒スルガ其褶襞ハ極メテ不規則ニ重疊スル

馬鈴薯ノ上ニハ不規則ナル凹凸ヲ有スル菓子狀結節ヲ形成スル

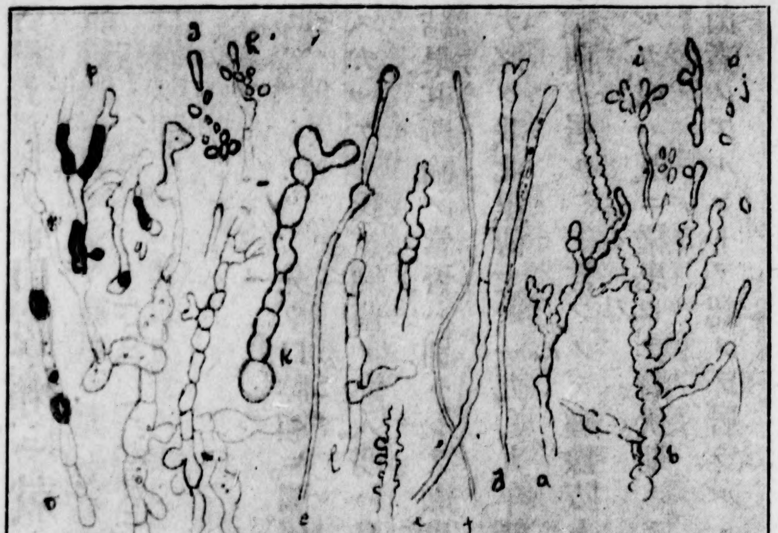


Fig.36 李種菌要素ノ顯微鏡的所見

菌學 安置培養法ノミデハ充分各種ノ形態ヲ見ルコトガ出來ナカツタソレデ安置培養ト斜面培養トヲ得タル菌學的所見ヲ綜合シテ記ス

菌絲ハ初メカラ分枝スルコトガ多ク且ツ其兩緣ハ平行デナク數珠樣ノ膨脹ヲ來ス(第三十六圖ab(然シ稍々舊イ培養ヲ見ルト兩緣ノ平行ナルモノモアル其際先端ノ膨脹ヲ呈スルヤウナノモ見ラレル(同c・d・e・f)是等ノ菌絲ニハ其側ニ數、多クノ枝ヲ出スガ其分枝ノ多キコト其形ノ奇ナルコトハ普通ノ白癬菌ニ餘リ見ナイ所デアル

外芽胞ノ發生ハ亦之ヲ見ル(g・h・i・j)其際 la migration protoplasmique ノ傾向モ亦之レアリ(g・j)

培養ガ舊クナルト菌絲ノ形ハ甚ダ變ジテ來ル或モノハ短キ圓形橢圓形ノ節ノ連鎖ヲ作ル(k)其節ヨリ亦不規則ナ側枝(同ジク短大ノ節ノ連鎖トシテ)ガ出ル(l・m・n)又菌絲中ニ立派ナクラミドスポールガ出來ル(o)尚ホ不規則ニ分枝セル菌絲中ノ或部分ニノミ染色可能ノ原形質ガ凝集シテ他部ハ色素ヲ取ラヌヤウナモノガアル(o・p・q)

是等菌學上ノ所見ハ總テノ大芽胞菌中最モ Tr. cerebriforme ノ菌學上ノ所見ニ近キモノデアル

動物試驗 Tr. néo-endothrix ニ屬スル菌種ハ本來動物試驗可能ノ筈デアルガ著者ノ場合數ヽモルモットヲ代ヘテ之ヲ試ミタガ常ニ不成功ニ終ッタ

鑑別 本種ガ大芽胞性ノ髮內外菌デアルトイフコト、其菌學的ノ所見ヨリシテ Tr. cerebriforme カ又ハ之ニ近キモノト思惟セラレル而シテ其培養基ノ外觀ハ此モノヨリモ寧ロ Tr. plicatile ニ近シト考ヘラル、ノデアル但シ本種ノ鑑別ハ向後尚ホ同樣ノ菌種ヲ得テ比較シテ見タ上デナケレバ確カニ斷定スルコトハ出來ヌノデアル

(未完)

最モ衣襞狀菌ニ近シト考ヘタニ止ル向後若シ同様ノ菌種ヲ得ルコトモアラバ其時比較ニ資センガ爲メニ此ニ簡單ニ記錄スル

培養ノ外觀ヨリ之ヲ見レバ本種ハ岩穴狀菌(時トシテ尖圭狀菌)ニ似ル然シナガラ其髮内外種ナルコト、菌學上ノ所見トニ於テ前兩種トノ間ニ逕庭ガアル又培養ハ常ニ純白色デ腦廻轉狀菌ノ如ククレーム色トナラヌ本種ニハ決シテ變態(プレオモルフイズム)ヲ見ナイ動物移植ニハ成功シナカツタ

臨牀的所見 第二十三例 李達新十歳男奉天平康里住・診斷頭部表在白癬兼疥癬大正八年七月四日診

疥癬ハ約半年來存ス頭部ノ白癬ハ何時カラ發生シタカ不明全頭部殊ニ顱頂部ニ當ツテ密生シ或ハ散在スル所ノ病竈ガアリ大ナル鱗屑及ビ脂漏ガ混ジテ厚イ膿痂ヲナス其周圍ニハ孤立シタ小病竈ガアル

鏡檢 主トシテ毛髮ノ外部ニ之ヲ圍繞シテ大型ノ芽胞及紐狀ノ菌絲ガアル毛髮内部ニモ紐狀又ハ數珠狀ノ菌絲ガアル卽チ鏡檢上ニハ寧ロ黄癬狀白癬菌ニ由ル病毛ニ似タル形狀デアツタ

培養 最初葡萄糖寒天ノ上ニ白色ノ小點ヲ作リソレガ增大スルニ從ツテ褶襞ヲ作リ其直徑ハ二糎位デ病毛ノ顯微鏡像ト併セ考ヘ白色黄癬狀白癬タラザルナキヤト疑ハシタ(插圖第三十二圖)

Fig.32 李種葡萄糖寒天第十二日培養

然シコレヨリ之ヲ葡萄糖寒天或ハ麴汁寒天ニ移植スルト發育良好ナル絨白色ノ絹樣光澤ノ表面ヲ有スル培養トナツタ初メハ尖圭狀菌ノ如ク中央尖圭狀ニ隆起シ且ツ放射線狀ヲナス深キ褶襞ヲ作ルガ後ニハ再ビ陷沒シテ其褶襞モ其形狀ガ不規則トナルノデアル

(第三十三圖ハ葡萄糖寒天上ノ第三十三日ノ培養第三十四圖ハ麴汁寒天上ノ第四十日ノ培養ヲ示ス)

斜面寒天ノ上デハ岩穴狀菌ニ酷似シタル培養ヲナス(第三十五圖)

三%ペプトン寒天ノ巨大培養デハ發育稍〻惡シク培養ノ直

Fig.33 葡萄糖寒天第三十三日培養

Fig.34 葡萄糖寒天第四十日培養

平等ニ侵サレルモノデハ無ク一腺胞ノ局部又ハ一葉ノミガ侵サレテ他ノ大部ハ比較的變化ノ無イコトガ多イ

各唾液腺中舌下腺顎下腺等ハ時々癩菌ニ侵サレル

唾液腺ハ最初ハ腫レテ一般ニ大キクナルモノ、樣デアル然シ其腫脹ノ凡テガ癩菌ノ侵害ニ因ルモノト看做スコトハ出來ナイ後期ニハ寧ロ萎縮スル又一旦侵サレテカラ病機ノ消退シタ殘跡ヲ見ルコトガアル卽チ之ニハ癩菌ハ絶對少數若クハ殆ド見當ラヌケレドモ一種ノ色素ヲ有ツタ上皮樣細胞ノ堆積及ビ其部腺組織ノ萎縮等ニヨッテ過去ノ病機ヲ想像セシメルノデアル

(五) 扁桃腺

扁桃腺ハ其組織的構造カラ觀レバ淋巴濾胞ノ如ク癩ニ侵サレ易イモノ、樣ニ想ハレルガ然シ屍體材料ノ病像デハ多クハ初期若クハ盛期ノモノデ此ニ陳舊ノ病竈ヲ見ルコトハ稀デアル夫レ故癩菌ノ進入門トシテハ菌ガ其處ヲ通リ拔ケスルヤ否ハ明カデ無イガ兎モ角吾人ノ鏡下ニ見タトコロデハ患者ガ癩ニ罹ッテカラズーット遲レテ或ハ周圍粘膜ガ一定度迄癩ニ侵サレテ後其結果トシテ病變ノ出來ルモノト見ルノガ穩當デアル

扁桃腺内ノ上皮細胞ヲ布麗シタ小管ニ癩菌ヲ見ルコトガ稀ナラズアル又多クハ濾胞ト同時ニ其部粘膜下小血管ノ瀰漫性浸潤ヲ見ルモノデアル

(六) 雜

舌其他ノ横紋筋纖維ハ概シテ癩菌ニ侵サレ無イ之ニ一二癩菌ノ進入スルコトハ絶對稀有ノ現象デアル又此場合デモ菌ハ此ニ增息シ得無イモノデアル

軟骨骨膜ハ屢、周圍ノ癩病機カラ連續的ニ侵サレル之カラ軟骨基質又ハ軟骨細胞内ニ一二癩菌ノ進入スルコトガアル神經管中軸索部ニハ癩菌ガ中心ニハ殆ドナク周圍ニ稍、多數ノ菌ガアッテ爲メニ横斷面ニハ小輪狀ニ觀エ縱斷面ニハ長ク絲狀ニ連續スルコトガ稀ニアル其他此ニハ菌ノ進入ト共ニ發育增息シ得ルモノト見エル

粘膜ノ上皮細胞ハ粘液腺ノ連續デアル點カラ觀テ此細胞内ニ癩菌ノ進入シ得ルモノト想ハレルガ然シ是ハ實際ニハ

殊ニ咽頭後壁等ハペルガメント樣滑澤トナッタモノガ顯微鏡下ニ見ルト粘膜下到ル處ニ極メテ菲薄ナル癩浸潤ガアッテ明瞭ヘマトキシリンエオジン染色デハ或ハ觀過スル的ノモノデアルニ拘ハラズ細菌染色標本デハ極メテ菲薄ナガラモ瀰漫性ナル癩菌含有細胞ノ浸潤ヲ見ルモノガアル

(二) 舌筋

舌筋ハ屢、單純萎縮ニ陷リ間質ノ鬆粗ナル結締織ニヨル補償的增息・脂肪組織ノ增加等ヲ見ルコトガアル此際興味アルハ神經細枝ガ甚ダ屢、癩菌ノ侵害ヲ蒙リ且ッ其侵害ガ普遍的ノコトガアル又神經終末體ノ侵サル、コトガ稀ナラズアル此際小血管ハ或ハ侵サレズ或ハ侵サレテモ輕イコトデアル蓋シ癩菌ガ舌ヲ侵スノ道ハ(イ)或ハ粘膜外カラ(ロ)或ハ周圍カラ連續的ニ(ハ)或ハ遠クカラ神經乃至血管ヲ介シテ病毒ヲ蔓延スベシト想ハレルノデアルガ就中(イ)ノ機轉ハ絕對ニ稀ナルベク(ロ)ニヨルモノ之ニ次ギ(ハ)卽チ神經若クハ血管カラ來ルノガ主ナ道デアラウ殊ニ終リノ二者ヲ比ベレバ場合ニヨッテハ神經ニ重キヲ置クベキヲ想ハシメルノデアル

(三) 粘液腺

口內粘液腺ハ周圍ノ癩浸潤ガ可ナリ著シイ場合デモ侵害サレ無イコトガアル反之鼻及喉頭ノ粘液腺ハ甚ダ強ク侵サル、コトガ屢、デアル是等ハ常ニ周圍ノ浸潤カラ連續的ニ來ルモノデアル其侵シ方ハ腺胞デハ間質カラ腺細胞ニ癩菌ガ進入シ排泄管デハ固有膜ニ先ヅ癩浸潤ガ出來夫カラ該部ノ上皮細胞ニ入リ此ニ寄生增息シテ彼ノ癩菌球ヲ形成スル夫レ故稍、太イ排泄管ニハ腺胞カラ來タモノト局部ノ周圍浸潤及ビ上皮細胞カラ來タモノトヲ管腔內ニ充實スルモノデアル

鼻粘膜ト口腔粘膜トヲ比ベルト鼻粘膜ノ方ガ粘膜及粘液腺共ニ遙カニ侵サレテ居ル喉頭粘膜ノ侵サレ方ハ屢、鼻粘膜ト伯仲ノ間ニアル

(四) 唾液腺

唾液腺ハ概シテ癩菌ニ侵サレ難イモノト見エル其侵サル、ヤ周圍カラ連續的ニ來ルモノデアル一ツノ腺デモ全腺ガ

第一例　元井　男　二十五歲　結節癩　大正五年十二月二十二日死亡

肉眼上所見　病變一般ニ高度デアル舌尖中央部同兩側緣ニ白色ニシテ上下ニ走ル陷凹シタ不正ノ瘢痕ガアル舌下腺ハ左右共腫大シテ居ル扁桃腺ハ少シク周圍平面カラ凸隆シテ居ル（輕キ肥大？）顎下淋巴腺ニ小豆大堅靭ナモノガ二ツ三ツアル咽頭粘膜ハ寧ロ萎縮ニ傾イテ居ル會厭軟骨ハ肥厚シタ部ト瘢痕短縮シタ部トアツテ爲ニ畸形ヲ呈シ前後面共ニデコボコデアル眞聲帶ハ左右共短縮腫脹シ其上下面共ニ瘢痕狀ニ見エル部ガアル喉頭粘膜ハ浸潤腫脹最モ著シイ披裂軟骨及其周圍ニモ高度ノ變化ガアル甲狀軟骨後面カラ氣管輪ニ亙ツテ粘膜ガ結節狀乃至浸潤狀ニ腫脹肥厚シテ居ル是等ノ變化ハ後壁ヨリモ前壁ニ著シイ

診斷　舌各所ノ小瘢痕・咽頭粘膜萎縮・喉頭粘膜劇甚ノ浸潤腫脹・聲帶ノ腫脹短縮・氣管上端前面ノ粘膜肥厚

組織的所見　舌　舌中央舌根等ハ其深部筋肉ハ輕度ノ萎縮ヲ示シ表在部卽チ粘膜ニ近キ方ハ高度ノ萎縮ニ陷ツテ居ル此部ハ筋纖維甚ダ細ク或ハ其太サ甚ダ不正トナリエオジンニ淡染シテ居ル然シ多クハ尚ホ橫紋ヲ目睹シ得ル此部粘液腺ハ大部分ハ缺損シ其各處ニ點々殘存スルモノハ却テ著シク增息シテ屢〻深ク筋束間ニ進入シテ居ル

癩病變ハ舌ノ粘膜上皮層下ニハ一般ニハ現ハレテ居ラヌ唯ダ各所散點狀ニ或ハ輕ク或ハ重キ變化ガアル其劇シイトコロハ局所的不正ノ浸潤トナリ若クハ結節狀デアル是等ハ恐クハ先ヅ結節ガ出來之ガ潰瘍ニ陷リ次デ瘢痕トナツタモノデアツテ其部ノ上皮細胞層ハ或ハ薄ク或ハ厚ク且ツ多クハ細胞核ノ染リガ良ク無イ病變部邊緣ハ時ニ其上皮細胞ガ深ク筋組織ニマデ進入シテ癌細胞索ヲ見ルノ觀ガアル其他筋層間ニモ各處ニ輕キ癩性浸潤竈ガ散在シテ居ル粘液腺ハ間質ノミノ癩浸潤ヲ呈シテ居ル病變ノ稍〻高度ナ部デモ其腺細胞內又ハ管腔內ニ癩菌ヲ寄生シタモノハ無イ

以上諸標本ヲ通ジ神經ガ每常侵サレテ中小神經枝及ビ神經終末體ニ多數ノ癩菌ヲ寄生シテ居ル之ニハ外神經鞘中往々少數ノ泡沫細胞ヲ作リ中部ニハ小癩球ヲ容レテ居ル是等神經ノ變化ハ殆ンド舌全體ニ亙リ普遍的デアル其他血管モ亦侵サレテ居ルケレドモ是ハ病變ノ程度ニ於テ劣リ又屢〻全然侵サレテ居無イモノガアル殊ニ動脈ハ勿論フライデアル又舌筋細胞內ニハ癩菌ハ見當ラヌ

舌尖　舌尖端カラ下面ニカケテノ切片ニモ筋纖維ノ萎縮ガアルガ此部筋纖維中ニハ纖維ノ太サニ變リハ無イガ染リガ惡クテ其橫紋ガ鮮明デ無ク爲ニ弱擴大ノ下ニハ硝子樣ニ見ユルモノヲ混ジテ居ル

神經ノ侵害ハ此ニモ普遍的デ稍〻大ナル神經枝ニハ外神經鞘ニ泡沫細胞ヲ作ツテ居ル其他終末體ノ末端ナル棍棒狀體ニ癩菌ヲ有ツテ居ルモノガアリ又神經管ノ軸索ニ適シ輪狀ニ排列スル癩菌ガアツテ此菌ガ二十ミクロンノ厚サノ標本ヲ二十枚迄連續的ニ同ジ太サデ追蹤シ得ルモノガアル

舌下腺　間質增息モ無ク腺胞モ侵サレテ居ラヌ

顎下腺　是亦癩菌ノ侵害ヲ受ケテ居ラヌ

扁桃腺　偏側ハ可ナリ侵サレテ居ルガ高度デハ無イ卽チ濾胞間組織カラ濾胞ニ亙ツテ癩浸潤ガアル上皮層カラ陷沒

絕對稀有ノコトデアル隨テ此細胞內癩菌ノ增息ハ絕無ト見テ可イ

甲狀腺ハ甚ダ癩菌ノ進入シ難イモノデアル但シ間質ニハ稀ニ(第一例第四例)絕對少數ノ菌ヲ見ルコトガアル

胸腺ハ癩菌ニ侵サレ難イ又同小體ニハ癩菌ヲ見無イ

口腔臟器癩病變及各部粘膜病程表

	第一例結節癩		第二例結節癩		第三例結節癩		第四例神經癩	
	粘膜及粘液腺	一般癩病變	粘膜及粘液腺	一般癩病變	粘膜及粘液腺	一般癩病變	粘膜及粘液線	一般癩病變
舌	+	筋間質及神經+	+	筋間質及神經+	+		+	筋間質及神經+
口蓋			+		++			
下鼻道			+++		+++			
懸壅垂					++			
會厭軟骨	++	骨膜+	++	骨膜+	++	骨膜+	++	骨膜+
咽頭	++						++	
扁桃腺	−	+		+	+	+	+	+
喉頭	+++		+++	骨膜+	+++	骨膜+	+++	
聲帶				++				+
氣管	+	軟骨−	++				++	
氣管枝					−	−		
食道			−				−	−
舌下腺		−		−				+
顎下腺		−		+		+		+
甲狀腺		−		−		−		−
淋巴腺及淋巴樣組織		++		++				

次ニ口腔癩四例ノ肉眼上及組織的所見ヲ記載スル

以下諸材料ハ凡テ十倍フォルマリン水貯藏ノ標本デアッテ之ヲ洗滌シテアルコホルニ移シタ後チエロイヂン封固ノ下ニ切片ヲ作リ明礬ヘマトキシリンエオジン染色ワンギーソン氏法及普通ノ癩菌染色標本ヲ作ッテ檢査シタモノデアル

ニ互リ廣汎ナル潰瘍ガアル鼻咽孔ハ酷ドク狹窄シテ居ル懸壅垂モ甚ダシク腫レテ大キイ會厭軟骨ハ肥厚短縮シテ居ル其前後面ニ潰瘍ガアル扁桃腺ハ別ニ腫レテ居ナイガ其面ニハ潰瘍(?)ガアルヤニ見エル喉頭入口ガ狹クナツテ辛フジテ鉛筆ヲ通シ得ル聲門ハ稍〻狹マツテ居ル氣管粘膜ニ淺在性潰瘍ガアル甲狀腺ニハ變リガ無イ

組織的所見 舌。各所ニ瘢痕的トナツタ舊イ小病竈ガアツテ菲薄ナル上皮細胞層カラ被ハレテ居ル此部上皮細胞ガ深ク筋組織間ニ進入シ時ニハ細胞索ノ末端ガ太ク肥厚シタ部ガアル是等粘膜表面ハ屢〻周圍平面カラ陷沒シテ居ル粘膜面ニ近キ表在筋織維ハ一般ニ萎縮シテ居ルガ深部ノモノハソーデ無イ癩浸潤ハ粘膜下一般ニ廣ガリ可ナリ高度デアル然シ上皮層ニハ癩菌ヲ見無イ極メテ稀ニ筋細胞內ニ癩菌小束ヲ容レタモノガアル此際筋細胞ニハ小裂隙乃至小管ガアツテ中ニ菌索ガアル樣ニ見エル又神經小枝若クハ其終末體內多數ノ癩球ヲ有ツモノガアル殊ニ神經小枝ノ癩菌ハ之ニ伴フテ居ル血管ニ菌ヲ見無イ際ニモ屢〻見受ケラレル

舌下腺　概シテ腺實質及間質共ニ變化ハ無イ癩菌モ見當ラ無イ

顎下腺　大部分ハ變化無ク間質ノ增息モ無イ然ルニ局部的ニハ著シキ癩變性部ガアル卽チ間質ノ癩浸潤デ腺胞內ニハ未ダ癩菌ヲ證明スルニ至ラ無イ又大小ノ神經ニ可ナリ多數ノ癩菌ガ居ルニ拘ラズ之ニ隨伴スル血管ニハ菌ノ無イモノガアル

一般粘液腺　粘液腺ニハ高度ノ變化ノ無イモノガ屢〻アル此場合デモ其何處カニハ局所的ニ間質ノ癩浸潤ガアツテ之カラ上皮細胞及ビ腺管內ニ癩菌ノ進入ヲ示スモノガ稀デ無イ

鼻粘膜及口蓋粘膜　軟口蓋中央部切片ニ就テ見ルニ口蓋粘膜ニハ肉眼上變化ガ無イニ拘ラズ組織的ニハ極メテ薄キ粘膜下癩浸潤ガアル之ニ對スル鼻腔面ノ粘膜下浸潤ハ可ナリ著シイケレドモ後方喉頭ノ病變ニ比ベレバ輕イ其粘液腺ハ約四分ノ一ハ侵サレテ居ル軟口蓋筋組織ハ可ナリ萎縮シテ居ル然シ筋細胞內ニハ癩菌ハ見當ラナイ

扁桃腺　病變ハ概シテ餘リ舊イモノデ無イ淋巴濾胞ハ凡テ高度ノ癩侵害ヲ受ケテ居ル之ニハカイムチェントレンヲ作リ到處癩菌又ハ癩球ガアル腺表面上皮ガ陷沒シテ出來タ管系統ガアル是等ノ管ハ其上皮細胞ノ成形元內ニ屢〻癩菌ノ寄生增息ヲ示シ時ニハ大ナル癩球ヲ作ツテ居ル然ルニ此標本ノ一部ニ接著シタ粘液腺ハ大部ハ癩菌ノ侵害ヲ受ケテ居無イ尤モ相互間ハ結締織デ隔テラレテ居ル又此切片中神經束ノ外神經鞘ニ著明ノ圓形細胞浸潤ガアツテ其內神經鞘細胞ニ無數ノ小癩球ヲ寄生スルモノガアル

會厭軟骨　前後面共ニ粘膜下ノ癩浸潤ガアル其後面卽チ喉頭ニ向ツタ方ハ殊ニ高度デアル此ニハ軟骨膜ノ浸潤ヲ起シ進ンデ癩菌ガ軟骨ノ基礎組織ニ入リ更ニ軟骨細胞內ニモ癩菌球一箇ヲ入レテ居ルモノヲ見ル然シ菌數カラ言フト周圍軟骨膜ノ浸潤部ニ比ベレバ軟骨組織中ノモノハ實ニ數百千分ノ一ニモ足ラヌ數デアリ其部位モ極メテ局限的デアル此部粘液腺ノ癩浸潤ハ實ニ高度デ先ヅ間質ガ侵サレ之カラ腺胞ノ基礎膜腺細胞內及腺腔內ニ癩菌ノ進入シ更ニ菌塊ヲ形成スルモノヲ少ナカラズ見受ケル

淋巴樣組織　咽頭ノ淋巴樣組織ハ癩ノ際一時肥大增息スルコトアルモノ、如ク是等ガ各處ニ癩浸潤ヲ受ケ無數ノ癩菌ヲ含シテ

シテ深部ニ管狀トナツタ部ニ癩球ガアル是ハ上皮細胞外ニ遊走シタ細胞ノ團塊ニ混ジテ居ルガ菌自己ハ細胞カラ離レテ孤立シテ居ル上皮細胞内ニハ癩菌ヲ見無イ

他側ノ扁桃腺ハ濾胞外緣ニ一定數ノ泡沫細胞ガアツテ此ニ少數ノ癩菌ヲ容レテ居ルケレドモ濾胞内深部ニハ菌ヲ見ナイ又粘膜ノ上皮層及上皮下ニハ全ク癩性變化ハ無イ

會厭軟骨中央部縱斷　前面即チ口腔面・後面即チ喉頭面共ニ高度ノ癩浸潤ガアル然シ浸潤層ノ厚サハ前面ノ方ハ概シテ薄ク喉頭面ノ方ハ甚ダ厚ク粘膜上皮細胞下カラ深部組織ニ波及シテ居ル粘液腺ハ喉頭面ノ方ニハ殆ンド見當ラ無イケレドモ時々癩浸潤内ニ同腺ノ面影ガ殘ツテ居ル口腔面粘液腺ハ間質ノ癩浸潤ニヨツテ腺胞ハ萎縮ニ陷リツ、モ尚多數殘存シテ居ル腺胞内又ハ排泄管内ニ癩菌ヲ見無イ

會厭軟骨自己ハ其軟骨膜ガ前面後面及尖端ノ三箇處ニ癩浸潤ヲ蒙リ殊ニ後面ノ浸潤ニ接シタ軟骨實質ニハ軟骨細胞内一二癩球ヲ容レタ部ガアル軟骨實質ハ萎縮ニ陷リ其厚サ甚ダ不正トナリ往々中斷シテ居ル

喉頭入口ノ後緣中央ヲ咽頭粘膜ト共ニ縱斷　之ニモ喉頭粘膜下ノ浸潤ガ強イ咽頭面ニモ薄ク廣キ膜下浸潤ガアル其他筋間質粘液腺間質ニモ癩浸潤ガアル又一部ニ靭帶ノ結締織化シタ部ヲ見ル入口部ノ中央尖端ニ結節狀ナレドモ周緣不正且ツ高度ナル癩浸潤竈ガアル

第三第四氣管輪(前方)　粘膜下ニ薄キ癩浸潤ガアル之カラ粘液腺間質ニ浸潤ヲ及ボシ爲ニ腺ハ多少萎縮ノ狀態ニアル腺胞及腺管内ニハ癩菌ヲ見無イ氣管輪軟骨モ無事デアル

淋巴腺　二箇ノ顎下淋巴腺ハ輕重程度ヲ異ニスル癩浸潤[illegible]居ル頸部淋巴腺ニモ亦一定度ノ癩病變ヲ呈シタモノガアル

甲狀腺　大部分ハ生理的デアル局部的ニ間質增息及腺胞萎縮?ニ陷タ部ガアル此部膠質ハ他ヨリモエオジンニ對スル著色ガ淡イ間質ニハ少數ノ癩菌束ガアル是ハ周圍結締織膜ノ輕微ナル癩浸潤ト連續シテ居ル膠質ニハ決シテ癩菌ハ見當ラヌ此[illegible]腫脹シタ淋巴腺ニ接觸シ該淋巴腺ハ可ナリ高度ナル[illegible]リ兩腺ノ皮膜ハ相互連接シテ居ルニ拘ハラズ此甲狀腺ガ[illegible]全ク侵害ヲ免ガレテ居ルノハ面白イ所見デアル

意見　此舌ノ癩病變ハ可ナリ陳舊ノモノデ恐クハ結節形成ヲ先驅シ之ガ潰瘍ニ陷リ後ニ瘢痕ヲ結ンダモノデアル此際大小ノ神經小枝ガ普遍的ニ侵サレテ居ル事ニヨリ菌ノ傳達ニハ主トシテ神經ガ關與シタノデアルマイカト想ハレル更ニ神經ト粘膜ノ病變ヲ比ベルト少クトモ粘膜病變ガ神經ノ夫レヨリモ舊イトハ言ヘ無イ咽頭ノ變化モ可ナリ舊イモノデアル扁桃腺ノ所見中其粘膜下浸潤ガ無クシテ濾胞ノ侵サレテ居ルノハ表面粘膜カラ直接ニ菌ノ進入シタノデ無イ事ヲ語ルヤニ想ハレル神經管ノ軸索又ハ終末軸索端(舌尖部)ニ癩菌ヲ見ルコト及會厭軟骨細胞内ニ少數菌ノ居ル事等ハ何レモ興味アル事實デアル

第二例　城西　男　三十六歳　病型結節癩　大正六年六月三十日死亡

肉眼上所見　一般粘膜ノ變化ハ高度デアル咽喉頭カラ氣管ニカケテ不潔ナル汚穢灰色ノマツセヲ容レテ居ル舌ノ變化ハ大シタコトハ無イヤニ見エル舌下腺ハ左右共ニ甚ダ大キイ顎下淋巴腺ノ米粒大ニ腫レ彈力性堅靭ナモノガ數箇アル下鼻道カラ鼻咽腔

居ル粘液腺ハ大部分ハ生理的デアルガ處々局處的ニ輕度ナル間質ノ癩浸潤ガアル

軟口蓋粘膜 瀰漫性ニ可ナリ高度ナル癩浸潤ガアル粘液腺モ亦可ナリ侵サレテ半バハ荒廢萎縮ニ陷リ半バハ間質ノ癩浸潤カラ延イテ腺細胞内及腺腔内ニ癩菌ノ進入發育ヲ見又管腔ニ癩球ヲ塡シテ居ルモノガアル排泄管モ亦略ボ同樣ノ病變ヲ呈シ之ニハ各處管腔ノ不正形ニ擴張シタモノガ屢〻アル此擴張ハ粘膜下結締織ガ高度ノ癩浸潤後ニ再ビ退縮シテ周圍カラ牽引シタ爲メト一部ハレテンチオンノ結果デアル深部筋層ハ概シテ萎縮ノ狀態ニアル

鼻粘膜 軟口蓋カラ下鼻道ニ掛ケテ一ツニ切ツタ標本ニ就テ觀ルニ鼻粘膜下ノ浸潤ハ實ニ高度且陳舊ノモノデ此部粘液腺ニハ淺在ノモノト深在ノモノトノ二種アルガ淺イ方ノモノハ甚シク荒蕪サレ大部分ハ消失シテ居ル其殘ツタモノハ腺ノ實質内ニ癩菌ノ進入增息ヲ示シテ居ル

鼻粘膜下ニ平面的ニ走ル筋及腱樣組織ガアツテ其下ニ深在粘液腺ガアル此腺モ多クハ廣汎ナル然シ新鮮ナル癩浸潤ニ罹リ間質腺細胞・管腔及排泄管ニ至ル迄多數癩菌ノ寄生ヲ示シテ居ル爲ニ部位ニ依ツテハ腺ノ肥大增息シタ部ガアル此種深部粘液腺ハ多クハ周圍ニ筋組織ガアツテ之カラ纏絡サレテ居ル此際筋組織ハ萎縮狀態ニアル斯ク深部ニ癩浸潤ノ出來ルニハ明カニ粘液腺排泄管ノ周圍結締織カラ深クニ及ボスノ狀ヲ見受ラレル然シ此深達ニハ他ニモ種々ノ徑路アルベキハ勿論デアル粘液腺侵害ノ程度カラ考ヘルト鼻粘膜ト口蓋粘膜ノ眞中位ノ部分ガ概シテ侵サレ方ガ最モ輕イ此深部粘液腺又ハ鼻粘膜下ニモ神經束内無數ノ癩菌ヲ含シ神經ガ癩菌ノ傳搬ヲ助ケルヲ想ハシムルモノガ屢〻アル極稀ニハ筋細胞内ニ癩菌索ヲ入レテ居ルモノガアル卽チ筋細胞ガ收縮シテ周圍カラ離レテ居ルニ拘ラズ其細胞中ニ菌ガ見エテ確カニ筋細胞内ニアルヲ示シテ居ル此際癩菌ノ周圍ハ特ニ透明ニ見エル又此種筋細胞ハ成形元ノ染色淡ク橫紋モ不正トナツテ居ル

其他此標本ニハ強靭ナ瘢痕樣結締織ガアツテ此ニハ不正三角形乃至不正形ナル結締織族細胞核ノ部ニ癩菌ヲ寄生シタモノガアル又腱組織中ニアル結締織細胞核ニ沿ヒ癩菌ヲ見ルモノガ各處ニアル

懸壅垂 不正形水腫狀ニ腫大シテ居タモノガ貯藏液ニ因ル收縮ノ爲メ表面ニ多少皺襞ヲ作ツテ居ル此標本ニハ粘膜下癩浸潤ガアル該浸潤ハ鼻粘膜面ノ方ハ瀰漫性デアルガ口腔粘膜ノ方及尖端ニハ結節狀ノ部ガ多イ此結節ハ屢〻神經末端ニ出來タモノデ圓形乃至類圓形ヲ呈シ健康部トノ境界ガ頗ルカツキリシテ居ル多數ノ粘液腺ガ各處局部的ニ侵サレテ居ル

顎下腺 分泌液ノ充實シテ腺細胞ガ透明ニ見エルノトハ反對ニ寧ロ腺ノ萎縮ニヨツテ各處竈狀ニ透明ニ見エル部ガアル是ハ殊ニ排泄管近圍ノ腺胞ニ於ケル所見デ多クハ其間質ニ色素ヲ有ツテ居ル其他ハ一般ニ實質間質共ニ大シタ變化ハ無イ前記萎縮部間質ニ極メテ少數ノ癩菌ガ處々散點狀ニ見受ラレル

右扁桃腺 中度ノ粘膜下浸潤ガアル濾胞ハ癩浸潤ノ結果トシテ今ヤ萎縮狀態(?)トナリ癩菌數ハ寧ロ稀少デアル上皮層ノ陷凹タル腺窩又ハ其管狀トナツタ部ニ無數ノ癩菌ヲ容レタ部ガアル此ニハ同時ニ多數ノ白血球ガアル然シ上皮細胞内ニハ癩菌ハ見

居ルノヲ見ル

喉頭　喉頭ニハ結節狀ナル粘膜下ノ癩浸潤ヲ初メトシ殊ニ粘液腺ノ荒廢ガ著シイ腺ノ一部ハ單純萎縮ノ狀デアルガ其大部ハ間質ノ高度ナル癩浸潤カラ進ンデ腺上皮細胞ノ成形元內及ビ內腔ニ癩球ノ多數ヲ有スルモノガ到處ニアル

眞聲帶ハ左右共全然瘢痕トナリ僅ニ痕跡ヲ止メテ居ルガ組織的ニハ周圍ト共ニ高度ノ癩病變ヲ呈シテ居ル此部ニアル纖維樣軟骨ニハ軟骨膜ノ浸潤及ビ骨基質竝ニ軟骨細胞內ニ至ルマデ癩菌小球ヲ有ツタ部ガアル更ニ其一小局部ニハ軟骨膜ト軟骨實質トノ境界ガ不明瞭トナツテ相互移行シテ居ル癩菌數ハ他部硝子樣軟骨ニ比ベレバ多ク又軟骨小體內ノ菌塊モ大キイ

モルガニー氏竇ノ粘膜變化ハ他喉頭粘膜ノ夫レト略ボ同一程度デアツテ其粘液腺ノ癩變化及ビ崩壞モ亦甚ダ著明デアル腺上皮細胞內及ビ粘膜上皮ノ陷沒シテ管狀トナツタ部ノ上皮細胞內ニ癩球ヲ有ツタモノガアル

氣管　上端粘膜ノ癩性變化ハ可ナリ高度デ喉頭ノ夫レニ近イ

食道粘膜　全然侵サレテ居無イ

甲狀腺　大部分ハ變化ガ無イ局部的ニ腺胞ノ多數ガ小サクテ胞內無數ノ小橢圓形體ヲ容レ間質稍〻著シク增加シテ萎縮ヲ想ハシムル部ガアル此部間質及ビ膠樣質內共ニ癩菌ヲ見出シ得ヌ

本例摘要　喉頭ノ癩浸潤ハ實ニ酷ドイ咽頭扁桃腺ノ變化順次ニ輕イ舌ニハ癩結節後ノ瘢痕ガアル舌筋組織ノ萎縮ハ可ナリ著シイケレドモ筋細胞中ニハ何レノ部ニモ癩菌ヲ見無イ粘液腺ハ舌ノ部デハ多クハ侵サレテ居無イ然ルニ喉頭咽頭ノ同腺ハ甚ダシクヤラレテ居ル本例デハ扁桃腺ノ凹窩ニ於ケル上皮細胞

內ニ菌ガ進入シ更ニ窩內ニ菌ノ出ル狀態ガ認メラレル其他モルガニー氏竇ニモ同樣ノ所見ガアル興味アルハ筋細胞內(舌)及軟骨實質內(會厭軟骨・聲帶部軟骨)ニ癩菌ノ迷入ヲ見ルコトデアル

第三例　田中　男　四十三歲　結節癩兼肺結核　大正六年五月十六日死亡

肉眼上所見　口腔臟器ニハ一般ニ癩性變化ガアル然シ前半部ハ侵サレ方ガ輕イ舌ノ大サハ通常・肉眼上大シタ變化ハ無イ軟口蓋粘膜ハ少シデコボコデアル鼻咽孔ハ狹窄シ下鼻道粘膜ニ癩浸潤ガアル懸壅垂ハ甚シク肥厚シテ居ル咽頭粘膜ハ稍〻萎縮シテ居ル扁桃腺ニハ索狀ノ結締織ガ網工ヲ作リ各處ニ凹窩ガアル其表面ハ粘膜面ト竝行乃至稍〻陷沒シテ居ル會厭軟骨ハ著シク腫脹シ喉頭粘膜モ不正浸潤狀ニ腫レ左右披裂軟骨ハ周圍ト共ニ畸形ヲ呈シ軟骨ハ結締織化(?)シテ居ル聲帶ハ短縮肥厚シ偏側前端上面ニ小潰瘍ガアル氣管分枝部ノ粘膜ニ粟粒大小圓形ニシテ周緣肥厚セザル多數ノ小エロジオンガアル其上方氣管ノ部ニハ毫モ變リハ無イ甲狀腺ハ稍〻大ニシテ質ガ硬イ大サ左葉四・〇—二・〇—〇・七糎重サ一一・八瓦右葉三・八—一・八—〇・八糎デアル(此葉ハ一部缺損ノ爲メ秤量セズ)舌下腺及顎下腺ハ左右共ニ普通ヨリモ稍〻小サイ

組織的所見　舌根及舌背　舌根大乳頭下ニ神經ニ沿フテ出來タ大病竈ガアル此ノ病竈ハ神經ニ沿フテ深ク筋間ニ迄進入シ然カモ周圍健康部トハ嚴重ニ境シテ居ル粘膜下ニハ極メテ菲薄ナ明礬ヘマトキシリン・エオジン標本デハ一寸判ラ無イ程度ノ癩浸潤ガ處々ニアル筋層ハ中度ニ萎縮シ其缺ヲ結締織デ補充シテ

ニ初マルノ稀ナラザルヲ示ス點デアル然シ此事ハ强ガチ粘膜ニ限ツタコトデ無ク自分ノ實驗デハ皮膚ニ於テモ其限局性結節ハ同ジク神經末器ニ初マルコトガ屢〻アル

氣管分枝部以下ニ癩病變ヲ來スコトハ絶對稀有デアル

第四例 多田 男 四十四歳 神經癩腹膜炎合併 大正五年十二月九日死亡

肉眼上所見 口腔内變化輕微一般粘膜萎縮ノ狀態デアル舌ニハ變化アル如キモ確カナラズ舌下腺ハ小サク（萎縮）顎下腺ニハ變化ヲ見無イ扁桃腺萎縮（？）咽頭粘膜萎縮滑澤・會厭軟骨後方粘膜面ニ糜爛シタ部ガアル披裂軟骨ハ周圍ト共ニ瘢痕狀ニ變形シテ居ル偏側聲帶前端ニ粘膜ノ缺損アルヤニ見エル氣管輪粘膜健常甲狀腺ニ異常ハ無イ顎下及頸部淋巴腺ニ小豆大質堅靭割面帶赤黃色ノモノガ二ツ三ツアル

組織的變化 舌 舌筋一般萎縮卽チ核ハ增加シ筋纖維ハ概シテ細ク成形元ハ多クハエオジンニ濃染シ或ハ却テ淡染シ往々斑紋狀ニ染マリ又時ニ龜裂ヲ見ル間質結締織ハ鬆粗ニシテ厚ク各處脂肪組織デ充塡シテ居ル癩浸潤ハ主トシテ上皮層下乳頭毛細管外膜ニ沿フテ現ハレ極メテ薄ク狹クシテ瀰漫性デアル菌ハ半ハ上皮樣細胞中テアリ半ハ癩球トシテ存シ其他少數ノ孤立シタ癩菌ガアル粘膜下ノ神經小枝又ハ其終末體ニモ屢〻癩菌ヲ見ル是ハ間質ノシユワン氏鞘細胞內ニ居ル舌筋內間質ニモ各處毛細管ニ沿ヒ又ハ神經小束內ニ散在性ニ極メテ少數ノ癩菌ガアル稀ニハ神經枝ノ中心ニノミ小菌塊ヲ容レテ居ルモノガアル是等ノ際該神經組織ハ屢〻其構造ニ大シタ變化ヲ呈シテ居ラヌ舌筋間質ノ癩變化ハ粘膜下ノ浸潤ニ續發シタモノデ粘膜下浸潤ノ稍〻高度ナ部位ニハ常ニ夫ニ續イテ筋質間ニ輕キ浸潤ヲ起シテ居ル

舌根部粘膜 此部粘膜下ニハ稍〻高度ナル浸潤ガアル是亦廣汎瀰漫性ニ上皮細胞層下ノ乳頭血管ニ沿フテ出來テ居ル粘液腺ハ萎縮シ腺細胞成形元ハ一般ニ濃染シ核モ濃染シテ假性增數ヲ示シテ居ル細胞ハ概シテ小サク各處腺胞ノ少數ガ其細胞核成形元質共ニ染リノ良ク無イモノガアル概シテ此部粘液腺ノ侵サレ方ハ大キナ舌乳嘴ノ下ニアルモノヨリモ夫レ以外ニアルモノ、方ガ稍〻著シイ癩菌ハ腺胞間毛細管ニ沿フテ各所ニアル又之カラ腺胞實質中ニ癩菌ノ進入シテ居ル部ガアル此腺病變ハ矢張リ粘膜下浸潤ノ續發ト見ルベキモノデアル

舌根部大乳嘴ノ下ニアル結節狀ナル神經終末體ニ一般ニ癩菌ヲ有ツタモノガアル之カラ連續スル神經小枝ニ引續キニ多數ノ菌ガアル是等癩菌ハ多クハ內神經鞘ノ細胞中ニ占居シテ居ル

舌下腺 大部分ハ單純萎縮ノ狀デアル時々全小葉ガ癩浸潤ニ陷リ又或ル葉ノ局部ニ稍〻高度ナル癩變性ヲ起シテ居ル

其小葉全部ガ侵サレテ居ルトキニモ之ニ接セル隣葉ハフライナ事ガ屢〻アル此侵サレタ葉デハ間質結締織乃至其毛細管內皮細胞ニ癩菌ガ居ツテ之カラ腺胞內ニ菌ノ進入スル狀態ガ見エル概シテ腺胞內癩菌ハ絶對少數デアル此際該小葉一般ニ單核圓形細胞ノ浸潤ヲ受ケテ居ル

小葉ノ局部的侵害ノ際ハ腺細胞ハ屢〻形態不正核染色不良ナルモノガアル又腺胞固有膜カラ上皮細胞內乃至上皮細胞ノ接合部ヲ通ジテ胞腔ニ菌ノ進入スル狀態ガ追蹤セラレル腺排泄管內ニモ亦固有膜浸潤カラ同樣ノ徑路ヲ取ッテ腔內ニ入イル狀態ガ見エル概シテ此種ノ新シキ局部性浸潤竈ニハ小葉全部ノ侵サレタ

當ラヌ唯ダ稀ニ菌ガ白血球ニ伴ハレテ上皮細胞層ニ入リ進ンデ上皮細胞内ニモ三四箇ノ菌ガ不正又ハ束狀ヲナシテ居ルモノガアル

○○○**會厭軟骨**　前面粘膜下ニ可ナリ劇シキ然シ薄層ノ浸潤型癩變化ガアル後方喉頭面ハ病變特ニ劇シク各處結節狀ナル厚キ浸潤ガアル是等ハ毎常神經小枝ニ沿フテ出來タモノデ周圍トハ頗ル鋭利ニ限界シテ居ル此部病竈ニハ中心ノ乾酪變性ニ陷ツタモノガアルラングハンス氏巨大細胞ハ見當ラヌ軟骨膜ハ一般ニ癩浸潤ヲ蒙ツテ肥厚シ軟骨自己ハ萎縮狀態ニアル後面下方ニハ骨膜侵害ノ結果軟骨基質内ニ束狀ノ癩菌ガ進入シテ居ル然シ軟骨細胞内ニハ菌ハ見エ無イ其他軟骨膜ノ癩侵害部カラ稍ヽ隔タツタ部ノ軟骨基質ニモ各處孤立シタ癩菌ガ點在シテ居ル粘液腺ハ此標本ニハ萎縮消耗ノ爲ニヤ見當ラヌ

○○○**喉頭入口後緣縱斷**　咽頭ニ向ツタ方ハ薄キ瀰漫性浸潤ヲ呈シ前面喉頭ニ向ツタ方ハ粘膜下浸潤ト共ニ結節狀圓形ノ病變ガアツテ會厭軟骨喉頭面ノ變化ト略ボ同樣デアルガ之ニハ粘液腺ガアツテ間質ノ癩浸潤ヲ示シテ居ル

纖維軟骨ノ一片軟骨膜ノ病變ト共ニ萎縮ニ陷ツテ居ル

假聲帶後部橫斷(上下斷)　此部ニハ粘膜下癩浸潤ノ他圓柱上皮ヲ布麗シタ腺管ガアツテ此管ヲ周擁シテ常ニ劇シキ癩浸潤竈ガアル腺管固有膜ノ癩浸潤カラ延イテ上皮細胞内ニモ少數ノ癩菌ヲ寄生シ更ニ管腔ニ菌ヲ容レタモノガ處々ニアル

氣管枝　氣管枝起始部ニアル粟粒大圓形ノ淺在潰瘍是ハ必シモ濾胞ニ適シテ出來タモノデハ無イ之ニハ癩菌ハ見當ラヌ軟骨輪ノ橫斷(上下斷)ヲ見ルニ凸面ガ内方ニ向ツテ居ル粘膜下粘液腺ニ癩菌ヲ見無イ然ルニ氣管枝軟骨輪ノ後方(外)ニアル數箇神經小束ノ一二ニ其外神經鞘内僅少ノ泡沫細胞ガアツテ之ニ少數ノ癩菌ヲ散見スル是ハ或ハ舊キ病竈ノ殘遺ト思ハレル

○○○**甲狀腺**　腺胞膠質ハ勿論間質モ亦變化ノ無イ處ガ多イ殊ニ腺ノ中心ハ概シテ生理的デアル腺ノ周圍ハ强靭ナル厚キ結締織ニヨツテ包マレ之カラ腺内ニ同樣ノ結締織束ヲ出シテ居ル是等結締織束ノ各處幼弱ナル肉芽組織カラ出來タ部ガアル此部ノ内容膠質ハ或ハ其膠化不充分ノ爲ニヤ染色淡ク或ハ顆粒狀或ハ不正小球狀トナツテ内方ニ退縮シ此ニ空隙ヲ殘シ或ハ反對ニ增息シタ壁ノ上皮細胞ニヨツテ全然内腔ヲ充實シタモノガアル此際上皮細胞ノ成形元ハ屢ヽ粘液細胞ノ如ク半透明ノ觀ヲ呈シ又ハ多數ノ空洞ヲ有ツタモノガアル斯ル場合往々同部間質ニ泡沫細胞ニ似タモノヲ見ルノデアル然シ確實ナル癩菌ハ其間質膠質内共ニ見當ラ無イ概シテ甲狀腺膠質ハ稍ヽ抗酸性デアルカラ癩菌染色標本ニ於ケル赤色孤立シタ桿菌樣ノモノデ屢ヽ癩菌デナイコトガアル爲メニ其辨別ニ大ニ苦メラレル兎モ角此甲狀腺ニ見ル多少ノ變化ハ或ハ癩ト何等カノ關係ガアルモノカ一寸判斷シ難イ

本例摘要　鼻粘膜及喉頭粘膜ノ侵害ハ甚高度デアル鼻粘膜カラ深部ニ癩病變ヲ及ボスニ當ツテ粘液腺ノ排泄管周圍ヲ介シテ菌ノ蔓延スル事ノアルノハ此例ニヨツテ確カデアル然シ他ニモ色色ノ徑路アルベキハ當然デアル

本例ニモ橫紋筋纖維内(軟口蓋)及會厭軟骨基質ニ絕對少數ノ癩菌ヲ見又上皮細胞内(扁桃腺部)一二ノ菌ガ迷入シテ居ル

此ニ興味アルハ粘膜下ニ結節狀圓形ノ癩病變ヲ發生スル場合邊緣ノ限界サレタル病竈ヲ作リ其病機ハ先ヅ神經小枝又ハ其末端

程度ハ上面ニ強イ之ニアル粘液腺モ侵サレテ居ル軟骨及聲帯組織自己ニハ癩菌ノ侵入ヲ見無イ

氣管輪 粘膜下ノ癩浸潤ガアル其程度ハ喉頭病變ニ比ベルト遙ニ輕イ然シ此ニアル少數ノ粘液腺ハ可ナリ萎縮荒蕪セラレ其僅ニ點々殘存スルモノモ必ズ多少トモ侵サレテ居ル但シ癩菌數ハ喉頭ニ比ベテ遙ニ少ナイ

食道粘膜 食道入口カラ三糎ノ部切片ハ粘膜及粘膜下組織共ニ癩性變化ヲ呈シテ居ラヌ其深部氣管輪ニ近キ部ニ輕キ癩性浸潤ガアル此ハ氣道粘膜及粘液腺ノ病變カラ氣管輪ノ間ヲ越エテ波及深達シタモノデ此間ノ連續ヲ明ニ認取スルコトガ出來ル

甲狀腺 其構造ニハ大部分ハ變リガ無イ然シ部位ニヨリ胞内膠質ノ充實度少ク周圍トノ間ニ間隙廣ク爲ニ屢々波紋皺襞ヲ作ツテ居ル又膠質内略ボ同大ナル無數ノ小楕圓形空虚部(空洞?)ヲ有ツタモノガアル或ハ膠質ヲ容レザル甚ダ小サナ腺胞ガ多數一處ニ集ツタ部ガアル但シ間質ニハ特別ノ變化ハ無イ是等ハ萎縮ノ狀態デハアルマイカ別ニ局部的ニ間質ノ少シク增息シ此ニ二三少數ノ癩菌ヲ見ル部ガアルケレドモ膠質内ニハ決シテ菌ハ見當ラ無イ此菌ハ隣接スル稍々大ナル靜脈外膜ノ癩浸潤カラ來タモノデアル

扁桃腺 表面ハ小窩ヲ失ヒ平滑トナツテ居ル鏡下ニハ粘膜下ニ極ク薄イ癩浸潤ガアル是ハ明礬ヘマトキシリン・エオジン標本デハ殆ンド觀過スル的ノモノデアル稍々深部ハ寧ロ瘢痕ニ近ク細胞ニ乏シキ強靭ナ結締組織トナツテ居ル此標本内ニアル粘液ハ主ニ單純萎縮ノ狀態デアル各處濾胞ノ肥厚增息及[illegible]菌塊ヲ有ツタ部ガアル是等濾胞内ニカイムチエントレン[illegible]テ居ル

結論

本例摘要 肉眼上咽頭粘膜ハ萎縮ノ狀態ニ陷リ粘膜面[illegible]滑ニシテ口腔内乾燥ノ觀アルモノデアル夫レ故組織内ニ[illegible]證明ハ恐クハ陰性ナルベシト思フテ居タ然ルニ上[illegible]ニ一驚ヲ喫シタ

蓋シ嘗テ高度ナル癩浸潤竈ヲ發生シ爾後病機ノ[illegible]粘膜ノ萎縮ヲ來シタモノト想ハレル

侵害程度ハ粘膜ニアリテハ喉頭ヲ優トシズツト輕イケレ[illegible]イテ舌根部咽頭粘膜舌一般及ビ氣管ノ順序デアル喉頭ハ[illegible]シク荒廢サレテ居ルニ反シ舌ノ病變ハ極メテ輕微ニシテ主ニ[illegible]純萎縮ノ狀態デアル粘膜以外ニハ唾液腺舌筋扁桃腺等ヲ侵シテ居ル然シ是ニヨツテ直チニ喉頭ヲ最舊ノ病變ト見ルコトハ出來無イ寧ロ咽頭舌粘膜唾液腺等ノ變化ガ或ハヨリ舊キモノデ無カラウカ(?)

本例ニ因ル他ノ小教訓ハ(一)筋細胞内ニ癩菌ノ進入スルハ甚ダ稀有ノ現象タルベキコト(二)神經纖維ノ軸索内(舌下腺)ニ癩菌ノ進入シタルコト(三)甲狀腺膠質内癩菌ノ寄生ハ恐クハ絕無ナルベキ事(四)癩菌ノ軟骨ヲ侵スヤ先ヅ骨膜ニ病變ヲ來シ續イテ其基質及細胞内會厭軟骨ニ癩菌ノ進入ヲ許スモノナルコト(五)粘液腺唾液腺ノ癩侵害ハ毎ニ間質ヨリ實質ニ及ブベキコト但シ唾液腺病變ハ概シテ輕キモノナルコト等デアル

時ヨリモ癩菌數ガ多イ

此舌下腺實質内深部ノ神經小枝又ハ稍〻大キナ神經束ニ癩菌ヲ寄生スルモノガ屢〻アル之ニハ神經束内平等ニ菌小塊ヲ入レテ居ル是等癩菌ハ主ニシユワン氏鞘ノ細胞内ニ居ルケレドモ時ニハ神經軸索ニ適シ其斷面ニ小輪狀ヲナシテ排列シテ居ルモノガアル概シテ舌下腺ノ癩浸潤ハ局所的ノモノハ勿論其一葉ヲ侵ス場合デモ粘膜下浸潤ノ連續ト見ルベキデアツテ周圍ハ常ニ稍〻高度ナル癩浸潤竈ニ續イテ居ル

○○○
顎下腺　腺實質ハ廣ク輕度ノ單純萎縮ニ陷リ爲ニ間質ハ半ハ鬆粗ナル結締織ニテ半ハ脂肪組織ニテ補償シテ居ル殊ニ腺小葉内排泄管ノ周圍ハ萎縮ノ度每常著シク組織ハ透明ノ觀ヲ呈シ細胞核ハ却テ淡ク然モカツキリト著色シ間質ハ特ニ增息シテ此ニ每常色素含有ノ結締織性細胞ガアル此色素沈著ノ多キコトニヨリテ排泄管周圍ハ他部ト大ニ觀ヲ異ニシテ居ル此色素ニハ鐵反應(?)ガ無イ癩菌ハ極メテ稀ニ間質ノ含色素細胞中乃至排泄管周圍ノ結締織中ニ散點シテ見エル

意見　顎下腺ハ舌下腺ニ反シテ其變化(萎縮)平等ニシテ癩菌ハ絕對ニ少數ナリ蓋シ該腺ノ變化ハ嘗テアリシ癩性變化ノ跡ト見ル可ク其含色素細胞ハ舊時ノ癩細胞ヨリ轉化セシモノニアラザルカ之ニヨリテ最初癩菌ノ進入スルヤ恐クハ排泄管周圍ヨリ間質ニ沿フテ來リシガ爲ニ此部ニ往時病跡ノ著シキヲ想ハシメ二ニハ舌下腺侵害ハ概シテ重カラズト雖病變ノ分布不平等ニシテ癩菌ハ却テ顎下腺ヨリモ稍〻多數ナルハ現存セル粘膜下浸潤ニ連續近遍スルニ因ルベキカ

○○○○
咽頭後壁粘膜　粘膜下癩浸潤ハ輕イケレドモ舌ノ夫ニ比ベルト

ヨリ高度デアル粘液腺ハ癩性浸潤ヲ蒙リ半ハ荒廢ニ歸シ一般ニ小圓形細胞ニ富ンデ居ル腺細胞ハ變形シ核ハ不正形トナリ[illegible]シ難イ是等腺胞内ニ癩菌ノ侵入スルアリ此侵入ハ[illegible]浸潤カラ來タモノデアル腺排泄管ニモ固有膜及管[illegible]癩菌ノ寄生增息ヲ見ル

○○○○○○○
喉頭入口及喉頭後壁粘膜　喉頭入口ノ後上端ニハ[illegible]浸潤ト共ニ結節狀ナル癩病竈ガアル此部ハ[illegible]シイ

喉頭後壁デハ粘膜下組織ヲ高度且ツ廣汎ニ[illegible]荒廢ニ陷ツテ居ル其腺ヲ侵スヤ[illegible]ニ於テ此方ガ優リ菌ノ數モ亦多イ[illegible]泄管ノ上皮細胞内大ナル癩球ヲ作リ[illegible]ニ管腔ニ落チントスルモノガアル

○○○○○
會厭軟骨中央縱斷　後方卽チ喉頭面[illegible]壁ト同樣デ可ナリ深部迄侵サレ主ニ血管[illegible]ナル浸潤デアル之カラ延イテ軟骨膜ノ癩性[illegible]軟骨基質中一二少數ノ癩菌ヲ見同時ニ軟骨[illegible]生シテ居ル

會厭軟骨前方卽チ口腔面ハ後面ヨリモ[illegible]層下ノ小血管ニ沿ヒ薄ク廣キ浸潤ヲ呈シ[illegible]半バ荒蕪サレテ居ル

○○○○○
環狀甲狀筋(?)　筋組織ハ廣ク萎縮狀態ニ[illegible]ナル結締織ト少量ノ脂肪組織ニヨツテ補償[illegible]ル粘膜ノ癩浸潤ハ可ナリ高度デアル

○○○○○
聲帶端部橫斷(上下斷)　上下面共ニ上皮細胞下ノ[illegible]

BIOLÓGIA, Bratislava — 24, 6, 1969

EFFECTS OF POLYENE ANTIBIOTICS ON PLANT, FUNGAL AND ANIMAL CELL MEMBRANES

Vladimír Betina, Helena Baráthová, Zoltán Baráth, Pavel Nemec

Department of Microbiology and Biochemistry, Slovak Technical University, Bratislava (Slovakia, S. S. R.), Department of Microbiology, Biological Institute, Slovak Academy of Sciences, Bratislava

Interactions of polyene antibiotics with fungal an
studied by many authors (1,2). Permeability alteratio
beet root *Beta vulgaris* var. rubra have been described
cation we wish to report differences observed in compa
polyene antibiotics, namely amphotericin B, nystatin
bility of plant, fungal and animal cell membranes.

Materials and meth

Polyene antibiotics

Nystatin was obtained from the Research Institute of A
(Czechoslovakia); flavofungin was a gift of Dr. I. Bék
University, Debrecen (Hungary); amphotericin B was a sa
which contained 50 mg of amphotericin B and 41 mg of
and Sons, New York). Stock solutions of antibiotics we
(DMSO) and stored at —10°.

Leakage of anthocyanins

From a beet root *Beta vulgaris var. rubra* 3 mm slices of a diameter of 1 cm were cut out and washed with water. Individual slices were put into duplicate sets of tubes containing each 6 ml of water supplemented with antibiotics. The final concentration of DMSO in all dilutions of antibiotics and in the controls was 1 %. The tubes were incubated in darkness at room temperature. Extinctions at 500 mμ were measured at intervals.

Leakage of anthocyanins from slices was estimated quantitatively using the method described by Nemec and Betina (3).

Leakage of riboflavin from E. ashbyi

A wild strain of *Eremothecium ashbyi* was used. From its sporulating cultures on slants of malt agar spores were washed out with distilled water and used for inoculation of 100 ml

BETINA, V., BARÁTHOVÁ, HELEN

Effects of polyene antibioti
animal cell membranes.

Biologia, Bratisl., Ser. C,
1 table. Slov., Russ. summ
Bratislava.

Microfilm no.

15

volumes of a complex medium 5 in 500 ml flasks. The flasks were incubated on a reciprocating shaker at 28° for 48 hrs. 2 ml volumes of the growing culture were used for inoculation of 100 ml flasks which were then incubated on the shaker at 28° for 36 hours. Various concentrations of antibiotics in DMSO were then added to the growing culture of *E. ashbyi* and the incubation continued for 6 more hours. The final concentration of DMSO in the media both in presence and in absence of antibiotics was 1 % (v/v). 5 ml samples were taken at intervals, cooled and centrifuged. Extinctions of supernatants were measured at 450 mμ and riboflavin concentrations were estimated from a standard curve. To the pellets, volumes of 5 ml of 5 % (w/v) trichloroacetic acid were added, the mixtures were homogenised, incubated for 30 min. in a water bath at 90°, and transferred into an ice-water bath for 20 min. After centrifugation the supernatants were used for estimation of mycelial riboflavin and in the pellets total protein was estimated according to L o w r y et al. using crystallized bovine plasma albumin (Armour Pharmaceutical Company, Eastbourne, England) as standard.

Lysis of human erythrocytes

Lytic effects of the three antibiotics on human erythrocytes were studied as follows. Fresh blood obtained from a volunteer was spun down for 10 min. at 2000 rev./min. using a bench ...resuspended in isotonic saline (0,9 % NaCl) buffered with ...8 and spun down again. Erythrocytes were gently dispersed ...2 to 4° before use.

...lition of 0,02 ml of the suspension of erythrocytes to 1 ml ...plicate sets of centrifugation tubes supplemented with anti- ... DMSO was 2 % (v/v) and it had no lytic effects.) After ...37° in a water bath tubes were spun down and extinctions ...50 mμ. When the same volume of the suspension of erythro- ...d water, it had an extinction of 0,5 at 550 mμ after total

1969
...ELENA, BARÁTH, Z. & NEMEC, P.
...iotics on plant, fungal and
... C, 24 (6):450-455. [3 graphs,
... summ. Slov. Techn. Univ.,
...5601

...lts and discussion

...hocyanins from root slices of *B. vulgaris* var. *rubra* ...esented on fig. 1. Flavofungin (100 μg/ml) was the ...Amphotericin B (100 μg/ml) after a lag of 2 hours ...hin the ...s flavo- ... ampho- ...tericin B caused a 100 % leakage of the pigments within 24 hours, while only 80 % of them leaked in presence of flavofungin at the same concentration. At a lower concentration (20 μg/ml) amphotericin B was much less effective

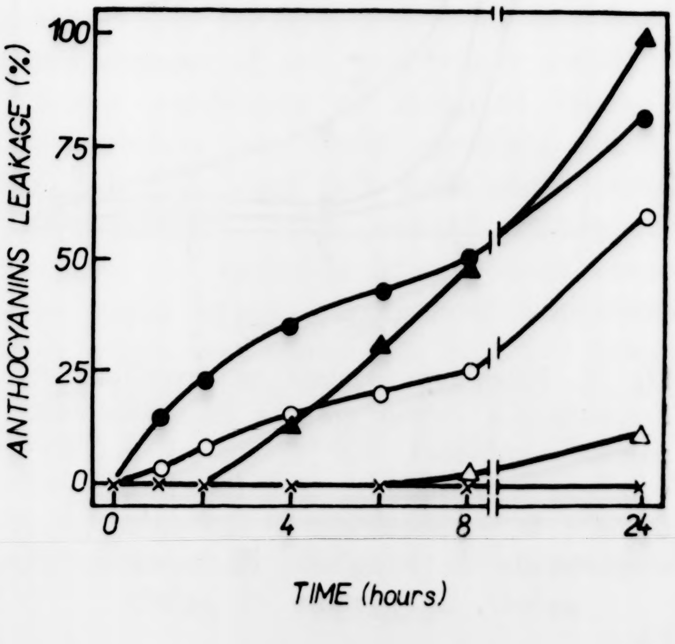


Fig. 1. Leakage of anthocyanins from root slices of *Beta vulgaris* var. *rubra* in presence of polyene antibiotics, ×, control; ●, flavofungin (100 μg/ml); ▲, amphotericin B (100 μg/ml); △, amphotericin B (20 μg/ml).

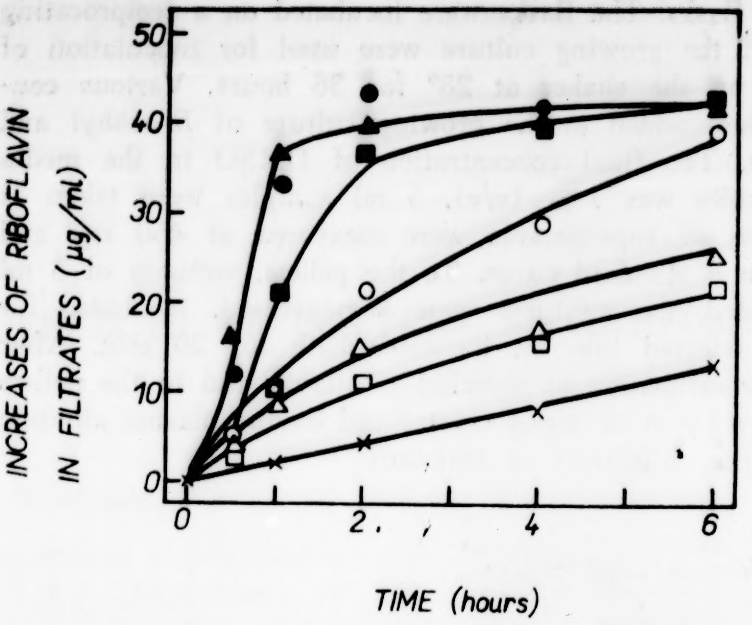


Fig. 2. Leakage of riboflavin from growing mycelium of *Eremothecium ashbyi* to media containing polyene antibiotics. ×, control; ▲, flavofungin (25 µg/ml); △, flavofungin (5 µg/ml); ●, amphotericin B (25 µg/ml); ○, amphotericin B (5 µg/ml); ■, nystatin (25 µg/ml); □, nystatin (5 µg/ml).

than flavofungin. Nystatin up to 200 µg/ml was ineffective.

Cell membranes of *Eremothecium ashbyi* were more sensitive to the effects of the antibiotics studied. The synthesis of riboflavin was stopped very soon after the antibiotics were added to the growing cultures.

Fig. 2 shows increases of riboflavin cencentrations in supernatants caused by antibiotics. The activity of the antibiotics was in the following order: flavofungin ≧ amphotericin B > nystatin. All antibiotics studied stimulated leakage of riboflavin from mycelia. Flavofungin and amphotericin B caused leakage of 96 % and 94 %, respectively, within 2 hours (fig. 3). Nystatin acted more slowly.

Changes of extinctions of supernatants indicating lysis of human erythrocytes caused by various concentrations of the three antibiotics are presented on fig. 4. Flavofungin at a concentration of 20 µg/ml caused a complete lysis within 5 min. but was inactive at 10 µg/ml. Amphotericin B at 20 µg/ml had similar effect as flavofungin, at 10

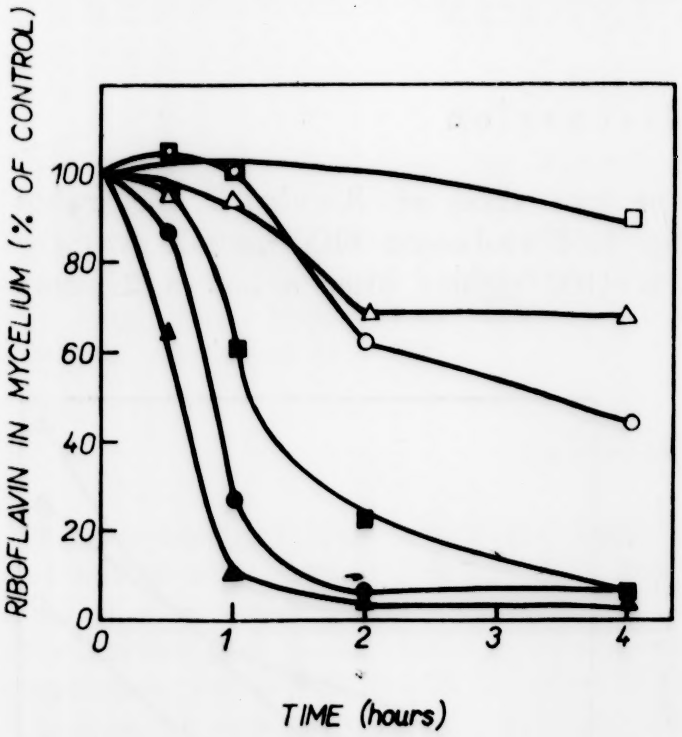


Fig. 3. Riboflavin content in mycelium of *Eremothecium ashbyi* growing in media with polyene antibiotics. 100 %, control; ▲, flavofungin (25 µg/ml); △, flavofungin (5 µg/ml); ●, amphotericin B (25 µg/ml); ○, amphotericin B (5 µg/ml); ■, nystatin (25 µg/ml); □, nystatin (5 µg/ml).

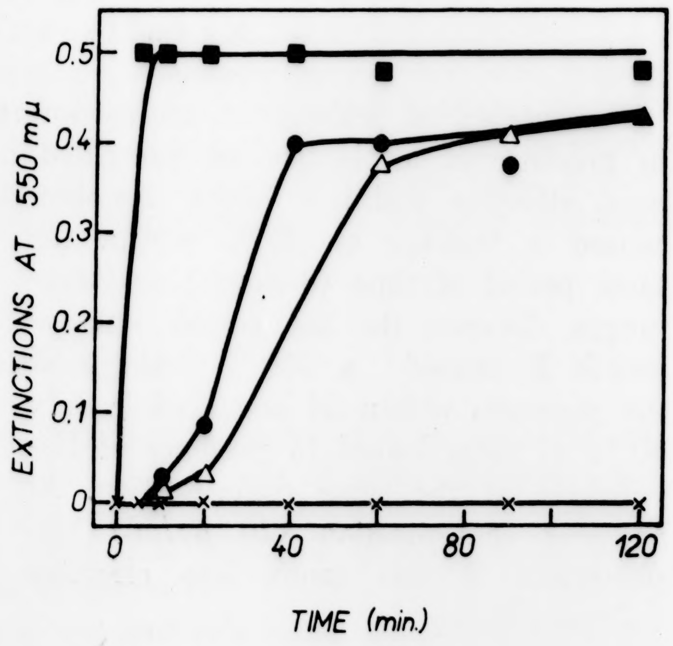


Fig. 4. Haemolysis of human erythrocytes by polyene antibiotics, ×, control; ■, flavofungin (20 µg/ml); ●, nystatin (80 µg/ml); △, amphotericin B (10 µg/ml).

μg/ml it had a lag of about 20 min., and was inactive at 5 μg/ml. Nystatin at 20 and 40 μg/ml was inactive, its effectiveness at 80 μg/ml was similar to that of amphotericin B at 10 μg/ml.

Flavofungin (4) is a pentaene antibiotic containing 31—32 carbon atoms in the molecule. According to Lampens classification it should belong to the group of „smaller" polyenes, amphotericin B and nystatin are members of the group of „larger" polyenes.

In our experiments flavofungin and amphotericin B were similar in their activities while nystatin was less active on erythrocytes and *E. ashbyi;* it was inactive in the case of *B. vulgaris* var. *rubra.*

We were able to demonstrate that human erythrocytes were most sensitive cells to lytic effects of the three antibiotics, followed by a lower sensitivity of *E. ashbyi.* Root cells of *B. vulgaris* were much less sensitive to flavofungin and amphotericin B and were not effected by nystatin up to the concentration of 200 μg/ml (tab. 1).

Table 1

Leakage of cell constituents caused by polyene antibiotics

Cell types	Cell constituents	50 % leakage caused by					
		flavofungin		amphoter. B		nystatin	
		μg/ml	time	μg/ml	time	μg/ml	time
Beta vulgaris var. *rubra* (roots)	anthocyanins	100 20	8 hrs. 23 hrs.	100 50[2]	8 hrs. 24 hrs.	200	—[1]
Eremothecium ashbyi (mycelium)	riboflavin	25 5	35 min. 200 min.	25	50 min.	25	75 min.
human erythrocytes	haemoglobin	20 10	5 min. —	20 10	5 min. 55 min.	80 40	35 min. —

[1] inactive concentrations,
[2] data from Nemec and Betina (3).

Permeability alterations induced by polyene antibiotics are ascribed mainly to binding of these substances to sterols in cell membranes (1, 2). In agreement with this theory qualitative and quantitative differences in sterol content of cell membranes should be responsible for different sensitivities to polyene antibiotics of different types of eucaryotic cells used in our experiments. Nevertheless one could speculate whether or not the presence and the composition of fungal and plant cell walls could protect somehow fungal and plant cell membranes (when compared with „naked" erythrocytes) against the effects of polyene antibiotics. However, the variation in sensitivity caused by the different physical state of the samples could be of even greater importance. With the beet root, leakage from 3 mm thick slices was measured, and the fungus formed balls of mycelium with possible restricted accessibility to the polyenes, whereas the erythrocytes are free cells. Thus a comparison of the sensitivity to polyenes of the three types of cells would not be warranted. On the other hand, one can conclude that flavofungin is the most potent, amphotericin B next and nystatin the least effective in causing leakage of cytoplasmic constituents of the three different cell types used.

Summary

1. Polyene antibiotics flavofungin and amphotericin B damaged cell membranes of beet root *Beta vulgaris* var. *rubra*. This caused leakage of anthocyanins from root slices which was measured quantitatively. Nystatin up to 200 μg/ml was inactive.

2. Riboflavin synthesis by *Eremothecium ashbyi* was stopped by these antibiotics and was followed by a rapid leakage of the vitamin from mycelium. Antibiotics were effective in the order flavofungin ≧ amphotericin B > nystatin.

3. Human erythrocytes were very sensitive to lytic effects of the antibiotics used. Nystatin had again the lowest activity.

We would like to express our thanks to Dr. I. Békési, Biological Institute, Medical University, Debrecen, for a sample of flavofungin. We also thank Mrs. L. Khandlová and Miss M. Sitárová for skilled technical assistance.

References

1. Lampen J. O., 1966: Interference by polyenic antifungal antibiotics (especially nystatin and filipin) with specific membrane functions. In E. F. Gale, Biochemical Studies of Antimicrobial Drugs. 111. Cambridge University Press, Cambridge.

2. Kinsky S., 1967: *Polyene antibiotics*. In D. Gottlieb and P. D. Shaw, *Antibiotics* I. Springer Verlag, Berlin. 122.

3. Nemec P., Betina V., 1968: Permeability alterations in beet root Beta vulgaris var. rubra caused. by arthotericin. B. J. Antibiot. (Seria A) 21, 626–627.

4. Úri J., Békési I., 1958: Flavofungin, a new crystalline antifungal antibiotic: origin and biological properties. Nature 181, 908.

5. Goodwin T. W., Pendlington S., 1954: Studies on the biosynthesis of riboflavin. Nitrogen metabolism and flavinogenesis in Eremothecium ashbyi. Biochem. J. 57, 631–641.

Authors' address: Doc. Dr. V. Betina, Ing. H. Baráthová, Prof. Dr. P. Nemec, Department of Microbiology and Biochemistry, Slovak Polytechnical University, Bratislava, Jánska 1. Ing. Z. Baráth, Department of Microbiology, Biological Institute of the Slovak Academy of Sciences, Bratislava, Jánska 1.

ÚČINKY POLYÉNOVÝCH ANTIBIOTÍK NA RASTLINNÉ, PLESŇOVÉ A ŽIVOČÍŠNE BUNKOVÉ MEMBRÁNY

Vladimír Betina, Helena Baráthová, Zoltán Baráth a Pavel Nemec

1. Polyénové antibiotiká flavofungín a amfotericín B poškodzujú membrány koreňových buniek červenej cvikly *Beta vulgaris* var. *rubra*. Prejavilo sa to uvoľňovaním antokyánov z koreňov, ktoré sa merali kvantitatívne. Nystatín bol neúčinný až do 200 μg/m.

2. Tieto antibiotiká zastavili syntézu riboflavínu kmeňom *Eremothecium ashbyi* a vyvolali rýchle uvoľňovanie riboflavínu z mycélia. Antibiotiká boli účinné v poradí: flavofungín ≧ amfotericín B > nystatín.

3. Ľudské erytrocyty boli veľmi citlivé na lytický účinok troch uvedených antibiotík. Nystatín mal aj tu najnižšiu aktivitu.

Dodané 4. 10. 1968

ВОЗДЕЙСТВИЕ ПОЛИЭНОВЫХ АНТИБИОТИКОВ НА РАСТИТЕЛЬНЫЕ, ЖИВОТНЫЕ КЛЕТОЧНЫЕ МЕМБРАНЫ И КЛЕТОЧНЫЕ МЕМБРАНЫ ПЛЕСНЕВНЫХ ГРИБОВ

Владимир Бетина, Гелена Баратова, Золтан Барат, Павел Немец

1. Полиэновые антибиотики флавофунгин и амфотерицин В повреждают мембраны клеток корня свеклы *Beta vulgaris* var. *rubra*. Это проявилось в освобождении антоцианов из срезов корня, измерявшихся количественно. Нистатин не был активен вплоть до концентрации 200 μг/мл.

2. Эти антибиотики приостанавливали синтез рибофлавина штаммом *Eremothecium ashbyi* и вызывали быстрое освобождение рибофлавина из мицелия. Антибиотики были активны в следующей очередности: флавофунгин > амфотерицин В > нистатин.

3. Эритроциты человека были весьма чувствительны к литическому воздействию трех названных антибиотиков. И активность нистатина была самой низкой.

Перевела А. Класова

1969

KRASSILNIKOV, N.A., KHODJIBAYEVA, C.M. & MIRCHINK, T.G.

Properties of toxins of Verticillium dahliae, the causative agent of Cotton wilt disease.

J. gen. appl. Microbiol., Tokyo, 15 (1):1-9. [3 fig., 1 graph, 1 table. Dept Soil Biol., Univ. Moscow.]

Microfilm no. 15802

J. Gen. Appl. Microbiol., **15**, 1–9 (1969)

PROPERTIES OF TOXINS OF *VERTICILLIUM DAHLIAE*, THE CAUSATIVE AGENT OF COTTON WILT DISEASE

N.A. KRASSILNIKOV, C.M. KHODJIBAYEVA AND T.G. MIRCHINK

Department Biology of Soil, University of Moscow, Moscow, Leninsky Gory U.S.S.R.

(Received May 11, 1968)

1. Determination of the toxicity of the culture liquid of *Verticillium dahliae* showed that it contains an active toxin which causes wilting of cotton plant shoots. Thus the fungus produces a toxin which it excretes into the external medium.

2. By testing a large number of strains of microorganisms from various taxonomic groups, a test microbe was found which is sensitive to the toxin of *Verticillium dahliae.*

3. Such a sensitive test-microbe made it possible to use chromatography with a bioassay method to select the best solvent for extraction of the toxin.

4. The toxin was obtained in concentrated form by extraction with amyl acetate.

5. A study of the physico-chemical properties of the toxin showed it to be acidic in nature, and that it has two sharp absorption maxima in the UV region at 245 and 275 mμ, and is stable at high temperatures and over a wide range of pH values.

6. The toxin of *Verticillium dahliae* is composed of two fractions, differing in physico-chemical and biological properties.

7. The toxin of *Verticillium dahliae* does not appear to be highly specific, and, besides its marked inhibitory action on cotton, causes wilting of leguminous plants, during which time cereals are not sensitive to the toxin in the concentrations tested.

8. The rate of wilting under the influence of the toxin depends on the age and variety of cotton plant. Adult plants wilt more quickly than young plants. The cotton plant variety 108-F (resistant to wilting) is less sensitive to the toxin than variety 2034, which is non-resistant to wilting. The toxin causes browning of the vascular bundles and the surrounding woody parts of the stalk, and the leaves loose their turgidity.

9. The tetraene antibiotics, 18–45 and 18–80, produced by actinomycetes, have an effect on the toxin produced by *Verticillium dahliae.* They interact with the fungal toxin and neutralise its action.

10. Treatment of seeds with antibiotics or cultivation in soils which have been enriched with the antibiotic-producing actinomyces produces an immunity in the cotton plants which is passed on to the following generations.

At present there is still no general agreement concerning the reasons for wilting of plants which have become infected by fungi. Among the earliest conceptions of this phenomenon, mechanical blockage of the vascular system by fungal hyphae or accumulation of toxic compounds formed by fungi in diseased plants was suggested as a reason for plant wilting (*1–3*). Recently, several authors have attributed such a role to glucosine (*4*). An important opinion is that plant wilting is due to active enzymes of the parasitic fungi which cause direct injury of tissues and disruption of cells (*5, 6*). More generally accepted at present however is the theory of intoxication, which states that plant disease is due to toxins excreted by the phytopathogen fungi into the external medium. A more detailed account of this phenomenon is to be found in the monograph by GAUMANN (*4*).

Many fungi are known to form different toxic products, some of which do not possess any kind of selective activity towards plant species (toxins with non-specific activity) and others which, on the contrary, are highly specific in their activity. Substances of a carbohydrate or protein nature, organic acids, and other compounds belong to the first group. Licomarazmin, a compound toxic to tomato plant, is an example of a specific toxin, being produced only by one fungus, *Fusarium lycopersici* (*7*). Victorin, isolated from the cultures of *Helminthosporum victoriae*, causes disease only in certain kinds of oats (*8*). A toxic substance towards sorghum has been obtained from *Periconia circinata* (*9*). Such toxins are essentially the cause of plant diseases. Their action on a given plant can reproduce all symptoms of a disease which would normally develop during a natural attack on the plant by the pathogenic fungus. They are agent of the disease.

However, the mode of action of such a toxin as fusaric acid (a compound produced by several species of the genus *Fusarium*, certain members of which are the agents of plant wilting) is somewhat different. With fusaric acid it is impossible to completely reproduce all symptoms of wilting, which fact suggests that the disease is not entirely due to the action of this acid (*10*). Also, the cause of cotton wilt disease by *Verticillium dahliae* is not fully understood. Lately, more and more evidence is accumulating which suggests that the wilting and destruction of cotton by *Verticillium dahliae* are also due to the toxin production by this fungus in the external medium (*6, 11, 12*). However so far there has been no information concerning the chemical nature and properties of these substances.

The elucidation of the chemical nature and biological properties of the toxins is of prime importance in solving the mode of action of *Verticillium dahliae* in the disease of the cotton plant, and in elaborating effective methods for the prevention of wilting. Thus, there is foundation for work demonstrating the toxin of *Verticillium dahliae* and studying of its physico-chemical properties.

METHODS

A culture of *Verticillium dahliae*, obtained from the Botanical Institute, Academy Sciences of U.S.S.R., was used in experiments concerned with the isolation of the toxin.

Toxicity of the cultural liquid of the fungus was determined on cuttings of the cotton plant, by submersion of the cuttings into the culture liquid. The culture liquid was obtained by growing *Verticillium dahliae* in malt extract medium for 5 days in 100 ml of medium contained in 700-ml flasks, which were shaken during the incubation period. After 5 days, the culture liquid was filtered and the cotton cuttings were submerged into it. In the control, cotton shoots were placed in tap water which had been acidified to the same pH as that of the cultural liquid (pH 5.0). The shoots were maintained in the culture liquid for 3 days during which time the rate of wilting was observed. Toxicity of the culture liquid was estimated from the rate of wilting of the shoots.

A paper chromatography method, used previously for antibiotics (*13*), was employed for selection of the best solvent for extraction of the toxin. The method of ascending chromatography was used, and included 14 solvent systems. To demonstrate the presence of the toxin on the chromatograms, a bioassay procedure with a test microbe was used. The concentrate obtained was studied with the help of electrophoresis and spectrophotometry. The UV spectrum of the concentrate was measured in a model SF-4 spectrophotometer. A pyridine-acetate buffer system (pH 5.9, 6.6, 7.4) in 30% acetic acid (pH 2) was used for the electrophoresis.

RESULTS

After 9–10 hr there were noticeable signs of wilting of the cotton plants which had been submerged into filtered culture liquid of *Verticillium dahliae*. The wilting became more and more acute as time passes. The leaves shrank, lost their turgidity, and wilted. After the second day the shoots were completely wilted, whereas control plants remained without change during this time. This fact suggested that there may be factors in the culture liquid which caused wilting. Work was then carried out with the aim of extracting the toxin which causes wilting of the cotton shoots.

The culture liquid was chromatographed and the best solvent was selected for subsequent extraction of the toxin. As the chromatograms were tested by a bioassay method with a test microbe, an organism had to be selected which was sensitive to the toxin of *Verticillium dahliae* and 125 strains of microorganisms from different taxonomic groups were tested; 30 species of bacteria and mycobacteria, 14 species of fungi, 33 species of actinomycetes, and one species of algae, *Chlorella*. From all of these microorganisms tested, only one culture, *Micromonospora vulgaris* strain 1005, proved to be sensitive to the toxin secreted into the culture liquid by *Verticillium dahliae*. The

results of chromatography of the culture liquid of *Verticillium dahliae* with the use of the test microbe *Micromonospora vulgaris* are shown in Table 1.

Table 1. R_f value of toxins in the different system of solvents.

Solvents	R_f
Acetone 80%	0; 0.9
Methanol-isoamyl acetate-water (24:14:1)	0.95
Butanol-hexane-acetone-water (20:9 10:1)	0.9
Acetone-benzene-water (30:9:1)	0; 0.6; 0.8
Distilled water	0; 0.8
Amyl acetate	0.3
Butanol-methanol-water (4:1:2)	0.9
CCL_4	0
Petroleum ether	0
Benzene, water-saturated	0.1
Ethyl acetate, water-saturated	0.5; 0.95
Chloroform, water-saturated	0.95
Butanol, water-saturated	0.5; 0.95
Isopropanol-chloroform-benzene-water (30:4:4:2)	0

The data obtained from chromatography of the toxin showed that this substance is very soluble in 80% acetone, ethyl acetate, chloroform, butanol, distilled water, and amyl acetate; only very slightly soluble in benzene, and insoluble in carbon tetrachloride and petroleum ether. In such systems as acetone-benzene-water and ethyl acetate-butanol, toxin is separated into two fractions. On the basis of the chromatography data, amyl acetate (R_f=0.3) was selected for extraction of the toxin.

Before extraction, the filtered culture liquid was acidified with 2 N HCl to pH 3.0. Without acidification, the toxin could not be extracted. One volume of amyl acetate was added to 2 volumes of culture liquid and the mixture was carefully shaken for 1 hr and the solvent was separated from the culture liquid. This was followed by a second extraction in which 1 volume of amyl acetate was added to 3 volumes of previously extracted culture liquid. The combined extract was then centrifuged at 1,500 rpm for 15 min and then filtered through filter paper to separate the emulsion which formed during the extraction procedure. The emulsion obtained in this manner, like the concentrate, was found to be active and was used for further investigations. It was soluble in water, ethanol, amyl acetate, and other solvents.

The concentrate was evaporated under vacuum at 40° to 1/100 of its original volume and tested for toxic activity by its action on the cotton plant and on the test microbe.

To test the activity of the toxin on the plant, a series of dilutions of the concentrate were prepared into which cotton shoots were introduced. To determine the activity against the test microbe, various dilutions of the concentrate were pipetted onto chromatography paper, which were then applied to an agar surface sown with *Micromonospora vulgaris*.

Results showed that cotton shoots were more sensitive than *Micromonospora* to the toxin. The concentrate was toxic to cotton shoots in a dilution of 1 : 1,000, and to the microorganism in a dilution of 1 : 20.

Certain physicochemical properties of the concentrate were examined. The toxin proved to be stable at high temperatures; boiling for 20 min did not reduce the activity of this compound. Changes in pH of the medium from 2 to 10 did not cause any fundamental change in its properties. With the help of electrophoresis it was established that the substance is acidic in nature.

The determination of ultraviolet spectrum of the concentrate showed the presence of two sharp absorption maxima in the region of 245 and 275 mμ (Fig. 1). As was mentioned above, in certain solvent systems (acetone-benzene-water, ethyl acetate, and butanol) there is a separation of the substance into two fractions, and these two fractions were eluted with amyl acetate.

First, the toxin was chromatographed with the solvent system of acetone : benzene : water (30 : 9 : 1). Each of these fractions was subjected to electrophoresis from which it was established that both compounds are acidic in character. On measuring the UV spectrum of the fractions, one of the latter,

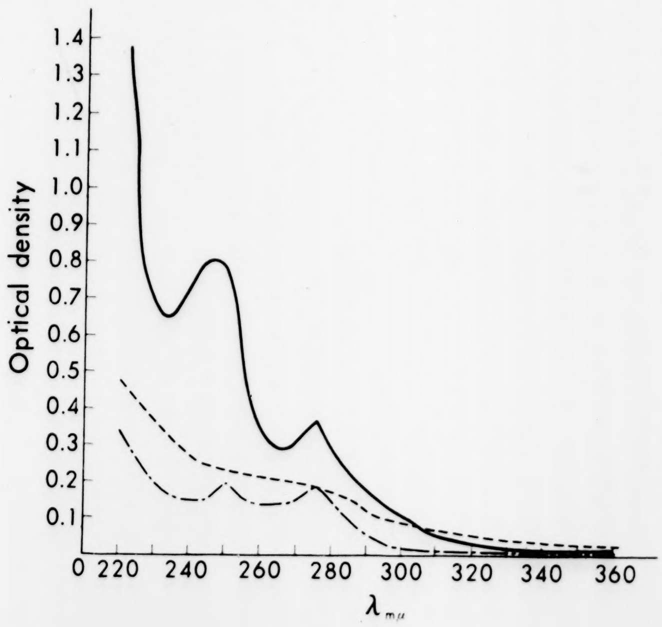


Fig. 1. UV absorption spectrum of the toxin (in alcohol).

—— common concentration

—·— fraction I

----- fraction II

with an R_f of 0.6, was found to have absorption maxima in the region of 250 and 275 mμ (Fig. 1). The other fraction, which had R_f of 0.8, did not have well defined absorption peaks.

The two compounds do not essentially differ from each other in solubility, and are stable at high temperatures and over a wide range of pH (from 2 to 10).

Action of Toxins on Plants

Effect of the two separated fractions of the toxin concentrate on the plant was then studied, with dilutions of the toxin concentrate in 1 : 10,000, 1 : 1,000, and 1 : 100.

It was observed that the rate of wilting depends on the age and kind of the cotton plant. Mature plants begin to wilt significantly earlier than young plants. Cotton plant Type 108-F (resistant to wilting) is less sensitive to the action of the toxin than Type 2034, a strain non-resistant to the wilt disease. Dilution 1 : 10,000 was almost without effect on cotton Type 108-F. Dilution 1 : 1,000 caused wilting within 9–10 hr. With dilution 1 : 100, which has a very marked effect on the plant, wilting developed within 5–6 hr. Cotton Type 2034 begins to wilt significantly earlier; *i.e.* within 18–19 hr at 1 : 10,000, within 5–6 hr at 1 : 1,000, and within 2–3 hr at 1 : 100.

When the plants were treated with the toxin from *Verticillium dahliae*, browning of the vascular bundles and the surrounding parts of the wood of

a b

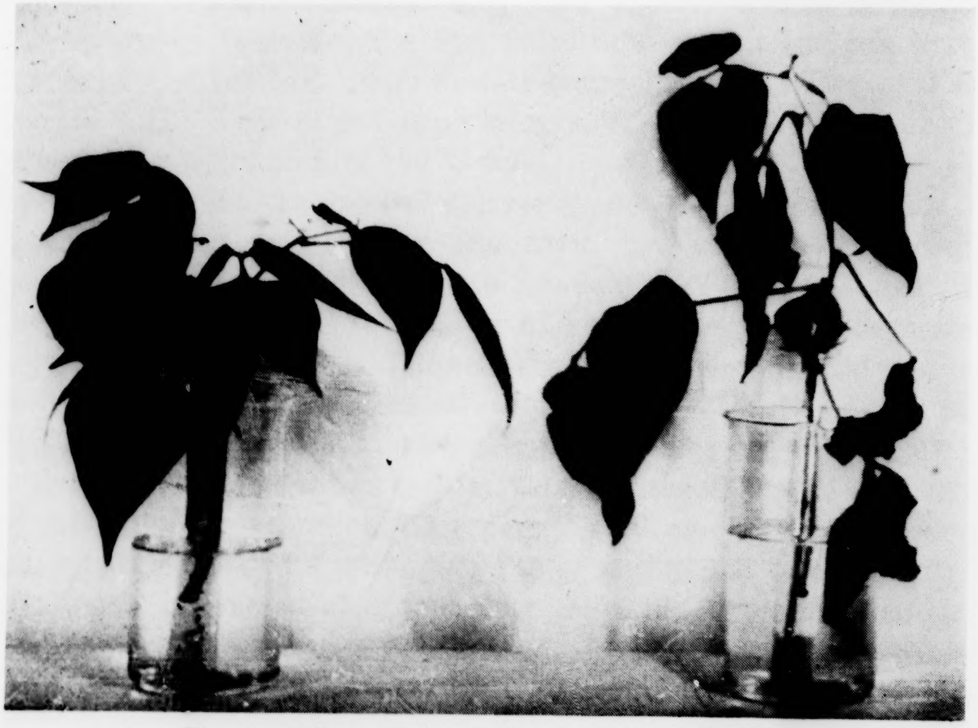


Fig. 2. Effect of the toxin on cotton shoots.

a) control b) solution of the toxin water

the stalk, and loss of turgidity of the leaves were observed. On testing the action of the separate fractions of the toxin, it was found that both substances caused changes in the plant, typical of the cotton wilt disease. Characteristic symptoms of the disease developed and the plants soon perished. The action of the toxin was tested on a series of other plants. When grown plants of cotton were treated with the toxin, it was absorbed by roots and entered in the stems and leaves, the highest concentration being in the roots, and lower in the stems and leaves (Fig. 3). In addition to the separation and study of the physicochemical properties of the toxin, antagonistic properties of actinomyces strains were tested, with the aim of finding an antibiotic which would be active against this fungus that causes the wilt disease. A large number of actinomycetes antagonistic to *Verticillium dahliae* were found. These antibiotics are polyene derivates. Two tetraene antibiotics, obtained from strains 18–45 and 18–80 and belonging to the group of "Albus" actinomycetes, were chosen for a more detailed study as they were highly active in relation to the prevention of cotton wilt disease (*14*). A determination was made of the ability of these antibiotics to inactivate the toxin formed by *Verticillium*

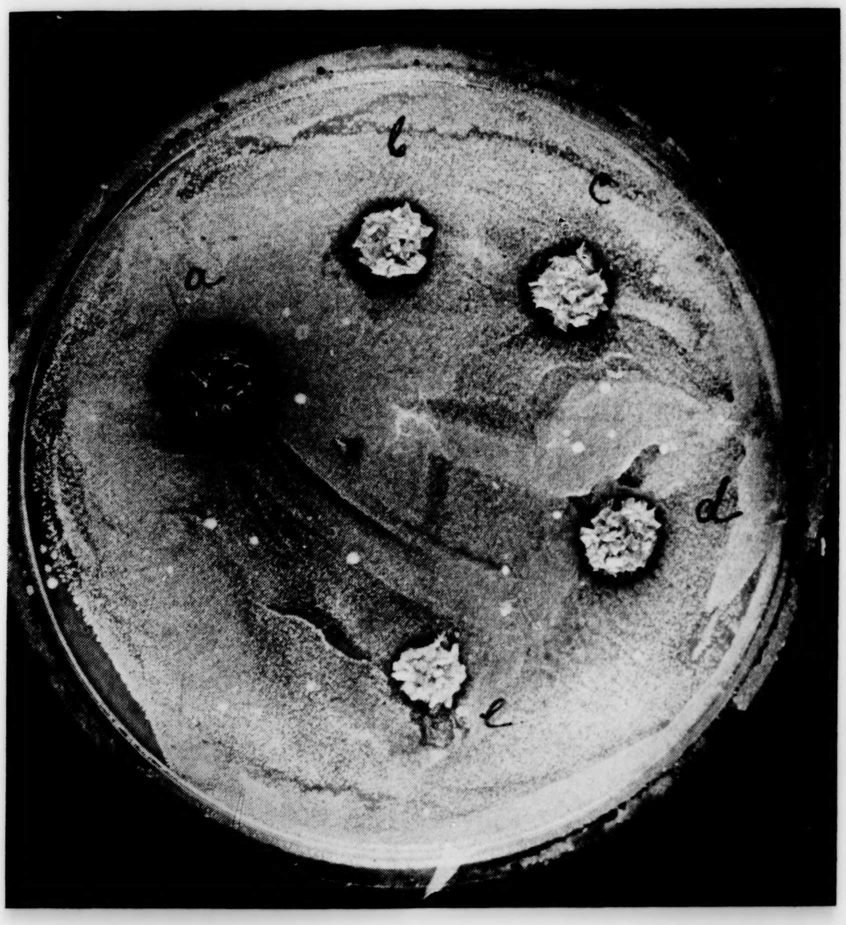


Fig. 3. Antibiotic activity of the plant treated with antibiotic 18–45 stem of the plant on a plate of *Micromonospora vulgaris*.

a) roots b, c) stem—lower part d) stem—higher part e) leaf

dahliae. One milliliter of the toxin was added to 1 ml of the culture liquid of Actinomycete (Strain 18–80 or 18–45), and this mixture was kept for 1 hr before being tested for toxicity against the cotton plant and the thermophilic actinomycetes, *Micromonospora vulgaris*. Two controls were set up, the first with cotton shoots submerged in a solution of pure toxin, and the second with the shoots in tap water. For the test on *Micromonospora vulgaris*, such a mixture was pipetted into a small cylinder on agar medium seeded with this organism. For the control, pure toxin was used without the antibiotic.

These experiments showed that the addition of crude antibiotic or its concentrate to the toxin completely eliminated the toxic action of the test solution. Plant treated with this mixture did not show any signs of wilting (Fig. 4). During the same period cotton shoots submerged in a solution of pure toxin showed definite signs of wilting, characteristic of the disease, and plants submerged into tap water remained healthy.

These antibiotics had a similar action in experiments using the test microbe. If a mixture of the toxin and antibiotic was placed on an agar medium seeded with *Micromonospora vulgaris*, no sterile zones of growth inhibition were formed during the time when pure toxin produced noticeable zones of growth inhibition.

Thus the antibiotics 18–45 and 18–80 not only inhibit the development of the causative agent of wilt, but also neutralise the toxin formed by this fungus.

Thus the cultures 18–45 and 18–80 produce a substance with double action; antibiotic and antitoxic. It is possible that there was a mixture of two metabolites in these preparations, as the latter was not particularly pure. It should be mentioned that the phenomenon of neutralisation of toxins of fungal and bacterial origins was observed by KRASSILNIKOV (*15*) with a series of antibiotics. Apart from their antibiotic and antitoxic properties, these

a b c

Fig. 4. Inactivation of the toxin by the antibiotic-antitoxin.

a) control b) effect of the toxin c) effect of toxin and antitoxin

antibiotics influence the immunity of the plant.

Cotton plants were cultivated in soil which had been previously enriched with actinomycetes composts, after which a determination of the fungicidal power of the cell sap of various organs of the mature plant was made. Crushed preparations of various organs of the plant were added to the centre of an agar surface, seeded with *Verticillium dahliae.* These experiments demonstrated that during the period when the sap of the control plants showed no fungicidal properties, plants grown in soil enriched with actinomycetes acquired these properties. Moreover, it was observed that plants grown from cotton seeds treated with the antibiotic were less susceptible to fungal infection.

Seeds of a cotton plant, which had previously been cultivated in a soil containing actinomycetes fertiliser, shows a normal growth. As a control, seeds of plants grown on ordinary soil without actinomycetes fertiliser were sown. After the plants developed to the 4–6 leaflet stage, the cotton plants were artificially contaminated with *Verticillium dahliae.* All the plants in the control acquired the wilt disease, while, when added with actinomycetes, only thirty-four per cent of the test plants was diseased with wilt. Thus, the use of these antibiotics enables the plants to acquire immunity in the following generation.

REFERENCES

1) G. SCHICCORA, *Arb. der Kaiserl. Biol. Aust. fur Land und Forstwirtsch.*, **5**, 157 (1906).

2) W.F. BEWLEY, *Ann. Appl. Biology,* **11**, 116 (1922).

3) B.A. RUDOLPH, *Hilgardia Journ. of Agr. Sci.*, **5**, (9), 201 (1931).

4) E. GAUMANN, Publishing House of Foreign Literature, Moscow. (1954).

5) S.S. GOTHOSKAR, R.P. SCHEFFER, T.C. WALKER and O. STAHMAYN, *Phytopathology,* **43**, 535 (1953).

6) M. KAMAL and R. WOOD, *Ann. Appl. Biol.*, **44**, N2, 322 (1956).

7) N. CLAUSON-KAAS, P. PLATTER and E. GAUMANN, *Ber. Schweiz. Bot. Ges.*, **54**, 523 (1944).

8) H.E. WHEELER and H.H. LUKE, *Phytopathol.*, **44**, 334 (1954).

9) R.P. SHEFFER and R.B. PRINGLE, *Nature,* **191**, N 4791, 912 (1961).

10) H.E. WHEELER, *Ann. Rev. of Microbiology,* p. 17 (1963).

11) P.W. TALBOYS, *Trans. Brit. Mycol. Soc.*, **40**, 415 (1957).

12) R.R. SHEFFER, S.S. GOTHOSKAR, C.F. PIERSON and R P. COLLINS, *Phytopathology,* **46**, 83 (1956).

13) N.O. BLINOV and A.S. KHOKHLOV, *Antibiotiki,* **2**, 183 (1962).

14) S.M. KHODJIBAEVA, *Microbiologiya,* **33**, No. 4, 661 (1964).

15) N A. KRASSILNIKOV, Academy of Sciences, Moscow (1958).

Apple-Packing Contests Again After Lapse Of Ten Years

REVIVED after a lapse of ten years, apple-packing contests proved a popular attraction when held again recently in the Roxburgh district. A crowd of more than 200 people filled the Apple and Pear Board store at Ettrick to watch the contests.

The events were staged by the Roxburgh Chapter of Jaycee in conjunction with the New Zealand Fruitgrowers' Federation Ltd. and the New Zealand Apple and Pear Board.

In the quality section, entrants were required to fill a case in three and a half minutes, wrapping and packing each apple from grading bins.

In the speed section entrants filled two cases as quickly as possible. This was won by B. Anderson in a time of 3m. 16s. Most of the profit of more than $30 will ... Jaycee project to assist a Volunteer Service Abroad team.

The Jaycees intend to hold this event again next year and hope it will be on a larger scale, possibly an Otago championship.

Results were:

Junior.—Miss H. Bagiery 1.

Intermediate.—W. Reichel, 171 points, 1; B. Breen, 167, 2; J. Stuart, 166, 3.

Senior.—D. Pearson, 176, 1; L. King, 175, 2; B. Pringle, 170, 3.

Speed.—B. Anderson, 3m. 16s., 1; Mrs. E. Tongahau, 3m. 23s., 2; L. King, 3m. 25s., 3.

Successful Observation Trials

OBSERVATION trials of Cox's Orange apples from export shipments has again proved very successful at Nelson.

The exercise, conducted for the first time last year, was to simulate export conditions so that at the end any faults not apparent before shipping could be ... of growers and this is a good thing," Mr. Baumgart said.

The plan was that each year fruit ... ers would be used ... the present rate it ... another three years ... s.

BAY OF

COOPER, KAREN, M. & MCKENZIE, D.W. 1969

Tree decline in older Apple trees on Malling XVI rootstocks.

Orchard. N.Z., 42 (4):133-136. [4 fig., 1 diag., Univ. Otago, New Zealand.]

Microfilm no. 15603

Moving int... rus and Chir... discussed, wit... charts, recom... for the vario... sub-tropical f... ture of the pr... tion for citru... in one directi... be obtained f... date. The ext... after a numb... in the form o...

Reduced cr... berries in a number of orchards is considered to be due to lack of sufficient pollination and one suggested method of overcoming this is to train a male vine at right angles across the rows above the top wire of the fences carrying the female vines.

Luncheon was provided by the N.Z. Fruitgrowers' Federation and was served from the packing shed of Mr. R. Earp at Te Puna. During the break a competition was held to identify specimens of pests, diseases and physiological disorders encountered on local fruit trees. This was won by Don Wills and Bill Baldwin of Te Puke who were each presented with a valuable book prize from the Associations.

The afternoon programme commenced with a demonstration of two blast sprayers provided by the Fruitgrowers' Federation, Auckland Branch, and Murray Pollett Ltd., of Tauranga.

Mr. W. Fletcher, Citriculturist, gave a very informative talk on the pollination requirements of citrus and sub-tropical fruits. This is a more important factor in the planning of new blocks of trees or vines than is generally realised, and Mr. Fletcher's talk will provide a lot of food for thought.

A discussion on a new technique of anchoring fence strainer posts was of particular interest to Chinese gooseberry and passionfruit growers. A fea-... which was explained ... Machinery Officer, ... culture, Hamilton, is ... of space at the end ...

... ectric Power Board ... emonstration of the ... hoist, normally used ... poles and high wires ... the removal of high ... g trees where they ... s.

... fternoon a wide var- ... ipment was on view ... itatives available to demonstrate and discuss their appliances. The interest shown by growers in this display would indicate that mechanisation of orchard operations is very much in their minds.

The day closed with an address by Mr. B. Piper, President of the Tauranga Sub-Tropical Fruitgrowers' Association.

... by Research Orchard, and at the Apple and Pear Board's depots at Mapua and Motueka so that all orchardists could inspect it to see faults for themselves.

"There was a very good attendance

PORT NELSON PROVED IT

GIVEN favourable conditions and the availability of fruit, Port Nelson had proved that 250,000 cases could be loaded aboard an overseas ship in four days, said the Nelson Harbour Board chairman, Mr. J. H. Brunt.

This loading rate was a result of the efforts of waterfront staff, suppliers and the Apple and Pear Board, he said.

'Tree Decline' In Older Apple Trees On Malling XVI Rootstocks

SEVERAL years ago, some unworked 10-year-old Malling XVI rootstocks at Havelock North Research Orchard were observed to have symptoms resembling flat-limb virus. In 1968, closer examination disclosed several features that are also commonly found in Gravenstein trees in New Zealand when they are infected with flat-limb virus already described by Chamberlain (1961).

by Karen M. Cooper* and D. W. McKenzie

The most obvious indications in Malling XVI stems were the typical flattened areas and large grooves in the main limbs (Fig. 1.a.) Less obvious and smaller sunken lenticular-shaped grooves were found in young branches. Very close observation led to the discovery of small dimples in quite young lateral branches. When the bark was stripped from the larger sunken areas, the wood beneath was seen to be darker yellow than normal. Instead of the usual smooth straight-grained surface, roughened areas were found tha twere finely indented with smaller pits. Concentric patterns were visible and suggested continual enlargement of the sunken areas with seasonal increments. (Fig. 1.b). This resembles flat-limb symptoms of Gravenstein previously described by McCrum et al (1960). In addition, when the bark was peeled from stems of the Malling XVI trees, the wood was often found to be dented by isolated small pits or small groups of pits. Exactly similar pits have been found in Gravenstein stems in New Zealand.

Fig. 1 (a) **Flattened areas on Malling XVI stem.**
(b) **Bark peeled from flattened areas on Malling XVI stem.**
(c) **Swollen node on Malling XVI stem.**
(d) **Bark peeled from swollen node on Malling XVI stem.**

Photos by J. W. Endt.

Concealed Symptoms Under The Bark

In addition to the grooves and pits in the wood, many stems of the Malling XVI trees had swollen and even bulbous nodes (Fig. 1.c). When the thickened, rather spongy bark was removed from these areas, the roughened discoloured wood beneath was found to be identical to that previously described (Fig. 1.d). A similar condition has been described as "swollen shoot" by Hovey (1963). This is also evident in most Gravenstein trees in New Zealand.

Identical With Gravenstein Flat-Limb Under The Microscope

As these symptoms appeared very similar to those already found in Gravenstein, it was decided to examine the Malling XVI limbs using the diagnostic anatomical methods developed in Australia on Gravenstein stems by Scurfield and Reinganum (1964). Microscopic studies showed that the first indication of the disorder in both Malling XVI and Gravenstein was a rapid tangential enlargement of ray tissue in the wood to about twice normal width and a partial loss of lignification of xylem elements. Continued expansion of the ray tissue forced vessel and fibres to undulate irregularly. Finally the wood tissues became completely disorientated and crumpled into irregular shapes. (Fig. 2.a).

Paired Vessels In Affected Wood

A unique characteristic was observed in the margins of grooved wood in both Gravenstein and Malling XVI stems. Vessels were found to occur in pairs. (Fig. 2.b.) Thee paired vessels were enlarged and distorted to form what appeared to be a series of unusual oval-shaped spaces in longitudinal tangential section. The adjoining vessels were divided by an almost invisble unlignified septum. In other instances, the pair of vessels were forced apart by a pene-

* Miss Cooper is a second-year undergraduate science student at the University of Otago who has spent the last two summer vacations working at Havelock North Research Orchard with Dr. D. W. McKenzie. She is the daughter of Mr. J. H. Cooper, a well-known fruitgrower of the Hastings district.

trating ray, giving the appearance of a small island of tissue suspended in an oval or even circular space. Very careful observation was required to see the thin cell wall between the two vessel elements. The paired vessels were always associated with the strongly disorientated thin-walled fibre tissue that has already been described above. Illustrations of the paired vessels from Gravenstein can be seen in Plate 4 Fig. 4 of the paper by Scurfield and Reinganum (1964), but their possible significance as a distinctive feature has not previously been discussed.

Tree Decline In Gravenstein

Flat-limb virus is a relatively slow-acting disease and in young Gravenstein apple trees there may be little evidence of disease apart from the deeply furrowed and twisted trunk. However, eventually, symptoms of tree decline appear, usually before the trees have reached 20 years of age. The first sign of disease appears in the most gnarled branches, which develop a pale leaf colour. The leaves of these branches progressively become more yellow. Fruit on affected branches ripens prematurely, often a week or more earlier than normal. Shoot vigour declines and fruit-size is reduced. Foliage becomes smaller in size, exposing the stems to sunlight and the bark often acquires an orange or yellow tinge. Eventually branches begin to die back and then the whole tree becomes stagnant. In this final moribund state, the tree usually succumbs to one of the root fungous diseases.

Fig. 2 Top photo (a) shows crumpled wood tissue of Malling XVI stem. The lower photo (b) shows paired vessels in wood tissue of Malling XVI stem. (Photos by W. A. Laing.)

"Dagger Cuts" In The Bark Of Gravenstein Trunks

Severe tree decline in Gravenstein is frequently accompanied by bark rupture of the major limbs. Raised blisters develop beneath the thick bark and may lift an area with a diameter as large as 8 ins. Each blister eventually ruptures, releasing a sudden flow of sap which may then ooze for several days. A short vertical slit is left, exposing the wood, and this is very slow to heal. More and more slits, resembling a series of "dagger cuts," appear as tree vigour declines. These are easily distinguished from normal expansion cracks in the bark which seldom expose the wood and are more extensively and irregularly disposed.

Tree Decline In Malling XVI

With some circumstantial evidence that Malling XVI might be susceptible to a long-acting unidentified disease, it was decided in 1969 to survey plantings of older trees on Malling XVI rootstocks in commercial orchards (in the Hawke's Bay area) and look for signs of tree decline. A dozen suitable blocks of apple trees were located by Horticultural Advisory Officers of the Departent of Agriculture who also assisted in collecting data. Scion varieties included Granny Smith, Jonathan, Cox's Orange, Red Delicious and Golden Delicious. Tree decline was evident in all bocks between 15 and 20 years of age that were examined. The course of the disorder showed the same sequence of external symptoms already described in Gravenstein, but with no obvious signs of trunk distortion in the scions. Pre-

Fig. 3 "Dagger cut" symptoms in the stem of Red Delicious on Malling XVI. (Photo by J. W. Endt.)

mature ripening of fruit was an indication of incipient tree decline. "Dagger cuts" symptoms were observed in the main branches of trees suffering severe tree decline. (Fig. 3.) This was most frequently found in Delicious trees, although all other scion varieties produced some trees with "dagger cuts." It was also noted that, in many trees showing severe tree decline symptoms, the short butt of the Malling XVI rootstock was deeply indented with cavities and in cross section these butts had

PROBABLY NOT A NEW DISEASE

APPLE growers should not become needlessly alarmed by this report and assume that this represents a new disease problem. It is probable that the tree decline condition has always been with us but not recognised as a possible virus disease.

The fact that for many years we have had a "replacement" problem in New Zealand orchards is a fairly good indication that apple trees have always had a limited period of useful production.

What in previous years has been called "wet-feet," "sour-sap" and simply "old age" would in some cases now be recognised as tree decline. Every effort will be made to resolve this problem and to offer a satisfactory solution.

similar ribbed outlines to Gravenstein trunks with severe flat-limb. (Fig. 4a.)

Not To Be Confused With Collar Rot

In the past, any trees with these "decline" symptoms have been suspected of having collar rot. However, when several of the worst of these trees were excavated, they were found to have sound roots with no evidence of collar rot. During the survey, only 2 per cent of the trees were found to be possibly infected with collar rot, athough all trunk butts were examined carefully for this disease.

Peeling Trunk Butts

For this purpose, pieces of bark with approximate dimensions of 1 in. x 2 in. were cut and peeled back from the trunk, and the surface of the wood beneath was examined (Fig. 4.b). Particular attention was paid to the bud union which in most trees was well above the ground level with the swollen butt of the Malling XVI clearly visible at the base of the trunk. In many instancts, the Malling XVI wood was discoloured and heavily grooved with small vertical furrows, and this was usually associated with trees showing severe tree decline symptoms. A milder symptom, with little or no discolouration of the wood and shallower, more dispersed furrows was associated in many cases with the early stages of decline. In all cases, the reverse of the identations in the wood showed up as wedges of phloem tissue on the inner bark surface. In one block of 125 Granny Smith trees furrowing was graded on a 1 - 4 scale according to severity. Observations showed that there was a close correlation between the severity of this furrowing and the state of decline of the tree. Details are shown in Fig. 5. Apart from a few trees with genuine collar-rot infection and some trees damaged by orchard implements, all trees with tree decline symptoms also showed moderate to severe signs of furrowing of the peeled wood in the Malling XVI rootstock butt. However, quite a few apparently healthy-looking trees also showed slight to moderate signs of furrowing in the wood. Evidently tree decline in the scion is preceded by this furrowed condition seen in the Malling XVI rootstock. It is notable that trunks of Gravenstein show identical grooves and pits in the scion wood, when the bark is removed.

Suspected Virus Disease

There is reason to suppose that a virus disease may be involved but this may be difficult to prove because of the long delay of up to 20 years in developing positive symptoms. Since some unworked Malling XVI trees produce symptoms resembling flat-limb, it is possible that the whole clone may be infected with this suspected virus. The serious nature of the tree losses in mature orchards, being caused by this disorder of Malling XVI rootstocks, cearly points up the importance of current work at Plant Diseases Division of the D.S.I.R. where an attempt to produce virus-free material of the main apple rootstocks and scion varieties is being made.

A1	B1	B1	B1	A1	A1
A1	A1	C1	E4	B1	A1
C1	A1	E4	C1	B1	B1
C1	A1	C1	E4	A1	B1
B1	C1	B1	B1	D2	B1
A1	E3	D1	C1	E4	D2
C1	E2	B1	E2	E3	C1
A1	B1	E2	D2	C1	E4
E3	A1	B1	A1	B1	B1
A1	A1	A1	E3	B1	E2
B1	A1	D1	B1	A1	C1
E2	C1	D1	D3	A1	A1
D1	C1	C1	A1	A1	B1
C1	E4	A1	D2	E3	E2
A1	B1	C1	D2	D1	D1
A1	C1	A1	B1	B1	C2
C1	A1	D3	B1	C1	C2
C1	C1	B1	E2	E3	D2
E2	B1	C1	A1	B1	B1
A1	B1	B1	A1	B1	B1
	C1	C1	A1	E2	B1

Fig 5 Incidence of stem-pitting symptoms in trunk butts of 20-year-old Granny Smith trees on Malling XVI in a commercial orchard where tree decline is developing.

KEY: A. No grooving. B. Trace of grooving. C. Slight grooving. D. Moderate grooving. E. Severe grooving. 1. Normal tree. 2. Slight yellowing of foliage. 3. Decline in vigour, small foliage, sunburnt reddish bark. 4. Severe decline, sick tree.

Fig. 4 In photo (a) on the left a trunk butt shows indentations and furrows in Malling XVI. (Photo by D. W. McKenzie.) In photo (b) on the right bark peeled from a Delicious trunk butt shows severe pitting and furrowing of the wood of the Malling XVI rootstock. (Photo by J. W. Endt.) Note the appearance of wood bendath "dagger-cut" in the Delicious scion.

Future Work

Survey work is proceeding in the search for other rootstocks that are not

(Continued on page 136)

affected by this tree decline condition, and if possible growers wil be advised as soon as alternatives have been found. Future recommendations will depend upon the choice of rootstocks that do not produce severe tree decline symptoms. It is to be hoped that many of the mature trees on Malling XVI rootstocks, approaching the critical age 12-15 years, can be rescued before the onset of tree decline symptoms, by inarching with more resistant rootstocks. The co-operation of fruitgrowers is sought in the search for tree decline symptoms in trees on rootstocks other than Malling XVI, and in particular on older trees on Malling XII and Merton 797 rootstocks. Horticultural Advisory Officers of the Department of Agriculture should be notified where cases of suspected tree declin have been located in such trees.

REFERENCES

Chamberlain, E. E. (1961). Virus Review of Fruit Trees. Supplement to The Orchardist.

Hovey, R. (1963). Note on the Swollen Shoot of Apple Trees; in Virus Diseases of Apples and Pears. Tech. Comm. 30. Commonwealth Agric. Bureaux.

McCrum, R. C.; Barrat, J. C.; Hilborn, M. T. and Rich, A. E. (1960). Apple Virus Diseases. Bull. Maine Agric. Exp. Sta. 595: 11-24.

Scurfield, G. and Reinganum, C. (1964). Some Anatomical and Chemical Effects of "Flat Limb" Virus in Apple Var. Gravenstein. Austral. J. Agric. Res. 15: 548-559.

RUSSIANS KEEN TO BUY FRUIT

RUSSIA is interested in buying fruit from Tasmania, source of most of Australia's apple crop, says a report from Canberra.

The main obstacle is an unfavourable trade balance, according to the Third Secretary at the Soviet Embassy in Canberra, Mr. Nikalai Poseliagin.

Australia was importing less than $2 million worth of goods from Russia annually, he said, while its wool exports to the Soviet Union were bringing in $25 million each year.

Tasmania is looking for new fruit markets following increasing competition in Europe, particularly from South Africa.

This year Tasmania will send more than 5 million cases of fruit to Europe, especially Scandinavia, and to South-East Asia.

Record Price For First Sales Of New Export Season

AN all-time record price for N.Z. Cox's Orange Pippins was realised at London's Covent Garden, at the first sales of the new apple and pear export season.

This popular variety fetched $8.60 per carton—about $1.50 higher than the prices realised last year.

Other varieties also fetched higher prices than last year. Kidd's Orange Red made $7.52 to $8.06, Jonathan $7.52 and Golden Delicious $7.31 per carton.

On the same day at an auction in the other major fruit market, Spitalfields, Cox's again fetched a high $8.60 for cartons of the smaller sizes and this price eased slightly to $8.38 to $8.54 for medium sizes. Golden Delicious realised about $7.40 per carton.

This fruit was of a consignment of 150,000 cartons of apples and 8,000 cases of pears carried by the *Laurentic*.

Early this month New Zealand apples and pears were continuing to fetch good prices on the United Kingdom market. In the face of increasing competition from South Africa and Australia our apples and pears were fetching above average prices for this time of the year.

For the period ending May 8 about 300,000 cartons of apples have averagd $6.23 per carton (c.i.f.), which is about $2 higher than the prices paid for a similar quantity sold at the same time last year. Pears are also fetching reasonable prices at around $3.56 a case.

"As Australia and South Africa intensify their exports to Britain during the next few weeks we can expect prices to even out, and the price our fruit is realising at present will drop somewhat," says the General Manager of the Apple and Pear Board Mr. D. L. Waller.

Efficiently Handled

"Meanwhile reports from our London Office say that the market in Europe is firm and that we can expect good returns from our first shipments to Hamburg and Antwerp."

TINNED FRUIT CUT DIFFICULT TO UNDERSTAND

THE reason given by the Government Stores Board for a 90 per cent cut in the supply of some varieties of tinned fruit to the Vincent Hospital Board caused amusement and annoyance at a recent Board meeting.

The Hospital Board was told its requirements of 240 large tins of greengages, nectarines and yellow plums had been reduced to 18 tins because suppliers could not deliver full orders due to frost damage in Central Otago.

One of the four fruitgrower members on the Board, Mr. G. R. Cambell, Alexandra, said the frosts must have been much heavier than growers realised. How Central Otago frosts could affect the supply of tinned greengages, nectarines and yellow plums was difficult to understand. Of all the fruit grown in the area, only apricots were tinned.

BETTER TO ALLOW IMPORT MONOPOLY FOR TROPICAL FRUIT

FRUIT Distributors Limited should continue to be sole importer of tropical fruit, the National Development Conference decided.

The chairman of the Conference's Distribution Committee, Mr. J. N. Laurenson, said the principle of this monopoly was contrary to all the committee's basic thinking. But the company had reached such a high degree of skill and efficiency in importing a most difficult and highly perishable commodiy that it would cost New Zealand a great deal to introduce competition in this specialised field.

The Distribution Committee considers that the negotiation of overseas supply contracts, shipping arrangemens, and the actual importation of tropical fruits are efficiently handled by Fruit Distributors Ltd. and that this company should remain, for the time being, the sole importing agency for imported tropical fruit.

It feels that such inequities and disadvantages as have occurred in the distribution of tropical fruit would lessen if not disappear if larger supplies of oranges, pineapples and other tropical fruit were allowed in through the removal of import licensing restrictions.

the bright sign of crop protection

FLIT 406
80% CAPTAN

Esso

PLANT FUNGICIDE

New strength FLIT 406 is proving its value for Orchardists all over New Zealand. Proving that protection and new economy mean top crops and top profits! Active ingredient—CAPTAN—is now 80% of total weight, and FLIT 406 is doing the job better, more economically, with less visible residues.

Marketed throughout N.Z. by all branches of THE NEW ZEALAND FRUITGROWERS FEDERATION LTD. for ESSO STANDARD OIL NEW ZEALAND LTD.

Methods of determination of the systemic and fungitoxic properties of chemicals applied to plants with emphasis on control of Verticillium wilt with Thiabendazole and Benlate*

D C Erwin†

A great surge of interest has arisen recently in the use of systemic chemicals for control of plant diseases. Methods for the detection of systemic chemicals and the principles underlying these methods are important to the manufacturer of chemicals who screens them and to the plant pathologist who determines their potential to control disease as well as their mode of action.

In 1966 the report of the systemic oxathiin compound 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin (Vitavax)® and its sulfone analogue (Plantvax)® for control of loose smut (*Ustilago nuda*) of barley and bean rust (*Uromyces appendiculatus*) by Von Schmeling and Kulka,[30] gave a dramatic boost to the confidence of plant pathologists in the efficacy of systemic chemicals for the control of certain plant diseases. The control of loose smut was particularly dramatic in that no externally applied fungicide had previously controlled the disease because the mycelium of *Ustilago nuda* was internally borne and could only be killed by heat. Even when a water bath was carefully adjusted to 54 C., the high temperature was often harmful to germination of barley seed.

In 1964 and 1966 Staron *et al.*[26,27] reported that Thiabendazole®, 2-(4-thiazolyl)benzimidazole (TBZ), a member of a new family of compounds (Fig. 1B), was fungitoxic and systemic in sugar beet plants and was capable of preventing Cercospora leaf spot (*Cercospora beticola*). In 1968 we reported that TBZ was taken up by roots of cotton plants, translocated to stems and leaves, and was of sufficient fungitoxicity to prevent symptom expression following inoculation of stems with spores of *Verticillium albo-atrum*.[15]

In 1968 another systemic benzimidazole compound, methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate (Benlate)® (Fig. 1A), was reported by Delp and Klopping[8] to prevent symptom exp as to control several oth caused by various meml scab caused by *Ventur* 1968 that Benlate appl wilt of cotton following of *V. albo-atrum*. The in leaves of plants was *lium expansum*.[13]

These recent finding ment of systemic fun Contrary to the experie reported previously,[7,10] severe with these benzi of those previously rep bility that fungal path pressed without harmin on the mode of fungitoxic action as well as mechanisms of translocation of these chemicals should open new leads to the production of even better compounds which might be more selective against many fungal pathogens.

Since Wain and Carter[32] so commendably reviewed many of the methods and principles involved with development of translocated systemic compounds, there is no need to review the literature here. Another recent compilation of work on systemic as well as other organic fungicides was prepared by Summers.[28] For this reason this discussion

ERWIN, D.C.
Methods of determination o
fungitoxic properties of c
plants with emphasis on co
wilt with thiabendazole an
Wld Rev. Pest Control, 8
1 diag., 4 tables,
Calif.]
Microfilm no.
15

*Based on an invitational paper presented at the First International Congress of Plant Pathology, in London, July 1968. A brief abstract was published.[11]

†Professor of Plant Pathology in the Department of Plant Pathology, University of California, Riverside, USA. The author wishes to acknowledge financial assistance by the Cotton Producers' Institute, National Cotton Council, and the Merck Chemical Division, Rahway, New Jersey, and the effective cooperation of Drs Isaac Malca, D. E. Munnecke, J. J. Sims and M. C. Wang and Mr T. A. DeWolfe and research assistants David Borum, Joseph Childers, Roger Harvin, Margaret Long, Henry Mee and James Partridge.

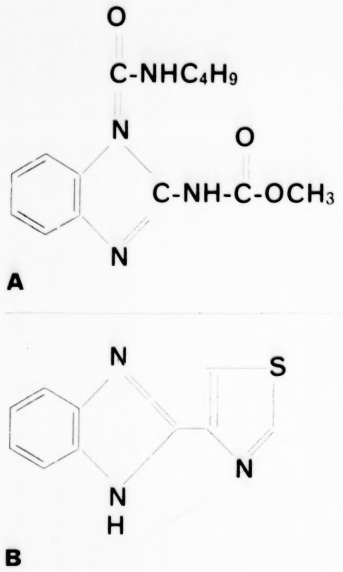


Fig. 1 A Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (Benlate)
B 2-(4-thiazolyl)benzimidazole (TBZ)

will emphasize examples from our research with the ben-
...it of others with the
...d compounds.
...st serious diseases of
...rains exist, a severe
...g strain.[21,25] In 1964
...mmended that a basic
...stemic chemicals for
...tton be made at The
This study was con-
...of natural genetically
...had not been found
...tum L.). Since 1964
...f growth regulators
...[2] as well as 300 other
...orted to be systemic
...neither TBZ nor Ben-
...ticillium wilt in the
...ents with TBZ and
Benlate encourage continued study.

1969
...ion of the systemic and
... of chemicals applied to
...on control of Verticillium
...le and benlate.
...1, 8 (1):6-22. [12 fig.,
...les, 33 ref. Univ. Riverside
5604

It is the purpose of this paper to elaborate principles and disciplines which will guide research in the field of detection of systemic fungicides. Emphasis will be placed on examples from our research on the control of Verticillium wilt of cotton caused by *V. albo-atrum* Reinke et Berth. (microsclerotial type), by the use of systemic chemicals. Limited literature exists on the control of this disease by use of systemic chemicals.[18]

Objectives of testing

One of the first criteria previously considered necessary for testing fungicides was *in vitro* fungitoxicity. Since some nonfungitoxic compounds become successful chemotherapeutants,[7] the well known *in vitro* agar and spore germination tests become less meaningful for the detection of a systemic fungicide than for contact fungicides. Also many highly fungicidal compounds are non-systemic. Therefore, *in vitro* tests cannot be primarily definitive for determining systemicity of a fungicide. Rather, determination of *in vitro* toxicity of systemic fungicides may be more important *after* the systemicity of the compound is established since from such data some information on the *in vivo* activity of the fungicide can be obtained.

Another important and limiting factor is the phytotoxicity of the chemical. Is the cure worse than the disease? Unfortunately this has been true in the past. Since the biochemical system of the fungal parasite has many similarities to that of a plant, phytotoxicity may perhaps always be one of the most important adversaries in the search for a successful systemic fungicide.

The most important factor in testing systemic fungicides is the effect of the chemical on disease expression. If effective systemic compounds could be either nonfungitoxic or fungitoxic, as Dekhuijzen[7] has pointed out, the effect on expression of disease is the most important and may be the only characteristic which is definitive. This theme was expressed numerous times by speakers on fungicides at the First International Congress of Plant Pathology in London. While testing the effect of chemicals on expression of disease is important, it is an expensive and time consuming process. Furthermore, such testing has been and may continue to be largely empirical until the data at hand are synthesized into workable theories that will relate chemical structure to systemicity and fungitoxicity.

Methods of testing fungicides in living plants

Since the effect of systemic chemicals on disease expression appears to be the only dependable primary screening practice, we have based nearly all of our research on systemic fungicidal control of Verticillium wilt of cotton on determination of the effect of chemicals on delay or prevention of symptom expression. For these studies we developed primary tests which are 'presumptive' indications of translocation of a chemical.

As Wain and Carter[32] stated, the first principle in determining the systemicity of a chemical is to place it at

Methods of determination of the systemic and fungitoxic properties of chemicals applied to plants with emphasis on control of Verticillium wilt with Thiabendazole and Benlate*

D C Erwin†

A great surge of interest has arisen recently in the use of systemic chemicals for control of plant diseases. Methods for the detection of systemic chemicals and the principles underlying these methods are important to the manufacturer of chemicals who screens them and to the plant pathologist who determines their potential to control disease as well as their mode of action.

In 1966 the report of the systemic oxathiin compound 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin (Vitavax)® and its sulfone analogue (Plantvax)® for control of loose smut (*Ustilago nuda*) of barley and bean rust (*Uromyces appendiculatus*) by Von Schmeling and Kulka,[30] gave a dramatic boost to the confidence of plant pathologists in the efficacy of systemic chemicals for the control of certain plant diseases. The control of loose smut was particularly dramatic in that no externally applied fungicide had previously controlled the disease because the mycelium of *Ustilago nuda* was internally borne and could only be killed by heat. Even when a water bath was carefully adjusted to 54 C., the high temperature was often harmful to germination of barley seed.

In 1964 and 1966 Staron *et al.*[26,27] reported that Thiabendazole®, 2-(4-thiazolyl)benzimidazole (TBZ), a member of a new family of compounds (Fig. 1B), was fungitoxic and systemic in sugar beet plants and was capable of preventing Cercospora leaf spot (*Cercospora beticola*). In 1968 we reported that TBZ was taken up by roots of cotton plants, translocated to stems and leaves, and was of sufficient fungitoxicity to prevent symptom expression following inoculation of stems with spores of *Verticillium albo-atrum*.[15]

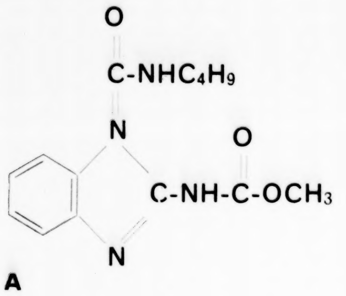
In 1968 another systemic benzimidazole compound, methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate (Benlate)® (Fig. 1A), was reported by Delp and Klopping[8] to prevent symptom expression of Verticillium wilt as well as to control several other diseases such as powdery mildew caused by various members of the Erysiphaceae, and apple scab caused by *Venturia inaequalis*. We also reported in 1968 that Benlate applied in soil prevented Verticillium wilt of cotton following inoculation of stems with spores of *V. albo-atrum*. The presence of a fungitoxic substance in leaves of plants was detected by bioassay with *Penicillium expansum*.[13]

These recent findings mark a new era in the development of systemic fungicidal control of plant diseases. Contrary to the experience with many systemic fungicides reported previously,[7,10] phytotoxicity has not been as severe with these benzimidazole chemicals as with many of those previously reported. There is an exciting possibility that fungal pathogens may be differentially suppressed without harming the host plants. Future research on the mode of fungitoxic action as well as mechanisms of translocation of these chemicals should open new leads to the production of even better compounds which might be more selective against many fungal pathogens.

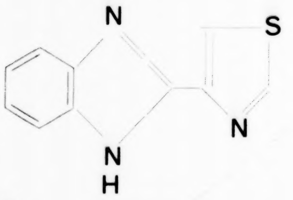
Since Wain and Carter[32] so commendably reviewed many of the methods and principles involved with development of translocated systemic compounds, there is no need to review the literature here. Another recent compilation of work on systemic as well as other organic fungicides was prepared by Summers.[28] For this reason this discussion

*Based on an invitational paper presented at the First International Congress of Plant Pathology, in London, July 1968. A brief abstract was published.[11]

†Professor of Plant Pathology in the Department of Plant Pathology, University of California, Riverside, USA. The author wishes to acknowledge financial assistance by the Cotton Producers' Institute, National Cotton Council, and the Merck Chemical Division, Rahway, New Jersey, and the effective cooperation of Drs Isaac Malca, D. E. Munnecke, J. J. Sims and M. C. Wang and Mr T. A. DeWolfe and research assistants David Borum, Joseph Childers, Roger Harvin, Margaret Long, Henry Mee and James Partridge.



A



B

Fig. 1 A Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (Benlate)
B 2-(4-thiazolyl)benzimidazole (TBZ)

will emphasize examples from our research with the benzimidazole compounds as well as that of others with the oxathiin and other recently developed compounds.

Verticillium wilt is one of the most serious diseases of cotton in the USA. At least two strains exist, a severe defoliating and a mild, non-defoliating strain.[21,25] In 1964 the Cotton Producers' Institute recommended that a basic and practical study of the use of systemic chemicals for the control of Verticillium wilt of cotton be made at The University of California, Riverside. This study was considered necessary because a high level of natural genetically controlled resistance to *V. albo-atrum* had not been found in American cotton (*Gossypium hirsutum L.*). Since 1964 our study has included the use of growth regulators (unpublished), systemic insecticides,[12] as well as 300 other compounds, many of which were reported to be systemic in the previous literature. Although neither TBZ nor Benlate has successfully controlled Verticillium wilt in the field, results of greenhouse experiments with TBZ and Benlate encourage continued study.

It is the purpose of this paper to elaborate principles and disciplines which will guide research in the field of detection of systemic fungicides. Emphasis will be placed on examples from our research on the control of Verticillium wilt of cotton caused by *V. albo-atrum* Reinke et Berth. (microsclerotial type), by the use of systemic chemicals. Limited literature exists on the control of this disease by use of systemic chemicals.[18]

Objectives of testing

One of the first criteria previously considered necessary for testing fungicides was *in vitro* fungitoxicity. Since some nonfungitoxic compounds become successful chemotherapeutants,[7] the well known *in vitro* agar and spore germination tests become less meaningful for the detection of a systemic fungicide than for contact fungicides. Also many highly fungicidal compounds are non-systemic. Therefore, *in vitro* tests cannot be primarily definitive for determining systemicity of a fungicide. Rather, determination of *in vitro* toxicity of systemic fungicides may be more important *after* the systemicity of the compound is established since from such data some information on the *in vivo* activity of the fungicide can be obtained.

Another important and limiting factor is the phytotoxicity of the chemical. Is the cure worse than the disease? Unfortunately this has been true in the past. Since the biochemical system of the fungal parasite has many similarities to that of a plant, phytotoxicity may perhaps always be one of the most important adversaries in the search for a successful systemic fungicide.

The most important factor in testing systemic fungicides is the effect of the chemical on disease expression. If effective systemic compounds could be either nonfungitoxic or fungitoxic, as Dekhuijzen[7] has pointed out, the effect on expression of disease is the most important and may be the only characteristic which is definitive. This theme was expressed numerous times by speakers on fungicides at the First International Congress of Plant Pathology in London. While testing the effect of chemicals on expression of disease is important, it is an expensive and time consuming process. Furthermore, such testing has been and may continue to be largely empirical until the data at hand are synthesized into workable theories that will relate chemical structure to systemicity and fungitoxicity.

Methods of testing fungicides in living plants

Since the effect of systemic chemicals on disease expression appears to be the only dependable primary screening practice, we have based nearly all of our research on systemic fungicidal control of Verticillium wilt of cotton on determination of the effect of chemicals on delay or prevention of symptom expression. For these studies we developed primary tests which are 'presumptive' indications of translocation of a chemical.

As Wain and Carter[32] stated, the first principle in determining the systemicity of a chemical is to place it at

a position on the plant remote from the place where disease expression occurs. For instance, if the chemical is placed on roots and the leaves are inoculated with a fungus such as *Botrytis fabae*, which causes chocolate spot of bean, a reduction in the numbers of lesions on the leaves is a presumptive indication that the chemical has translocated from roots and exerted an effect on the development of disease. This method has been used by Wain's group at Wye College and by others.[31] Essentially the same rationale would apply for study of powdery mildew or other diseases of leaves or stems.

The study of the vascular wilts is somewhat more difficult since the causal fungi naturally infect the roots and progress upward in the xylem by means of spores to the upper parts of the plant.[23] If the candidate fungicide is placed in the infested soil, the effect of the fungicide on disease expression could be strictly local and not due to the systemic property of the fungicide.

Dimond *et al.*[10] approached this problem by placing bare-rooted tomato plants in a solution or suspension of the candidate chemical, allowed them to take up the chemical for several days, after which the roots were washed free of the chemical and inoculated by dipping in a suspension of spores of *Fusarium oxysporum* f. *lycopersici*. A delay in disease expression and/or a reduction in severity of disease expression was used as a measure of the presumptive systemic effect of the chemical.

Although the root dip method was effective for inoculation by *Verticillium*, cotton plants suffered severe physiologic shock due to the bare rooting.[14] The growth of bare rooted plants which were dipped in water alone was reduced 50 per cent when compared to the controls. Also Keyworth and Dimond[19] indicated that injury caused by dipping roots could cause an increase in resistance to disease. Since these factors might be artefacts in assaying the effect of a chemical on disease control, other methods were developed.

Stem inoculation technique In an attempt to inoculate the plants at a point remote from application of the chemical, stem inoculation was employed.[14] This method was described originally by Brinkerhoff[2] and adapted later for inoculation of cotton in the field by Bugbee and Presley.[3] Dimond *et al.*[10] reported that stem inoculation of tomato plants with *F. oxysporum* f. *lycopersici* was relatively unsuccessful. The reason for this failure has not been explained. The most likely explanation would be that the inoculum drop does not reach the vascular tissue in tomato plants.

The methods described herein have also been briefly discussed in other publications.[13,15] Unless otherwise designated cotton (var. Delta Pine Smooth Leaf) plants were grown in steamed sand in 4-inch square polypropylene pots (Farrand Enterprises, Alhambra, Calif.). Plants were carefully selected for uniformity and grown in a greenhouse in which the minimum temperature was 24°C. and maximum 25–32°C. In growth chambers a 12 hour day (25°C.) and 12 hour night (18°C.) were used. Plants were fertilized twice weekly with a high nitrogen modified Hoagland's solution.[15]

The concentrations of all chemicals are based on the actual active ingredient of wettable powder formulations. TBZ applied was a 60 per cent wettable powder (Merck 01111W) and Benlate a 50 per cent wettable powder (DuPont 1991). *In vitro* data were from pure samples obtained from the manufacturers. ^{14}C-TBZ labeled at the bridgehead of the benzimidazole molecule was obtained from the Merck Chemical Division, Rahway, New Jersey.

Bud cell inoculum of *V. albo-atrum* was produced in a buffered (0·19M PO4) glucose–ammonium sulfate synthetic medium.[20] In a flask containing 10 ml of medium in an incubator-shaker at 25°C. approximately 4 billion spores in less than 5 days can be obtained. Although the buffered synthetic medium is most favorable for bud cell production, potato dextrose broth or Czapek's broth[4] can also be used. Concentration of inoculum (spores/ml) is estimated turbidimetrically.

Stems were inoculated with a droplet of spores on a 20 or 22 g hypodermic needle which was inserted through the bark into the xylem tissue (Fig. 2). Although the cotyledonary node on the base of the stem was most often selected, results of many experiments showed that any position from base to tip of the plant may be inoculated (Fig. 3). Inoculation of growing tips resulted in much delayed disease expression however. When three-week-old plants were inoculated, the first symptom was epinasty which was detectable in 3–5 days followed by chlorosis and necrosis of leaves. When the defoliating strain was used defoliation occurred in 6–10 days.

For quantitative control of inoculum density, the puncture in the axil of a petiole can be made with a dry needle after which a drop (0·01 ml) of inoculum can be applied with a micropipette. Since xylem tissue is under a negative tension the drop of spores is rapidly taken up by the plant.

The intensity of symptom expression can be varied by adjusting the concentration of inoculum. For instance when the number of spores in the inoculum was 5×10^5/ml,

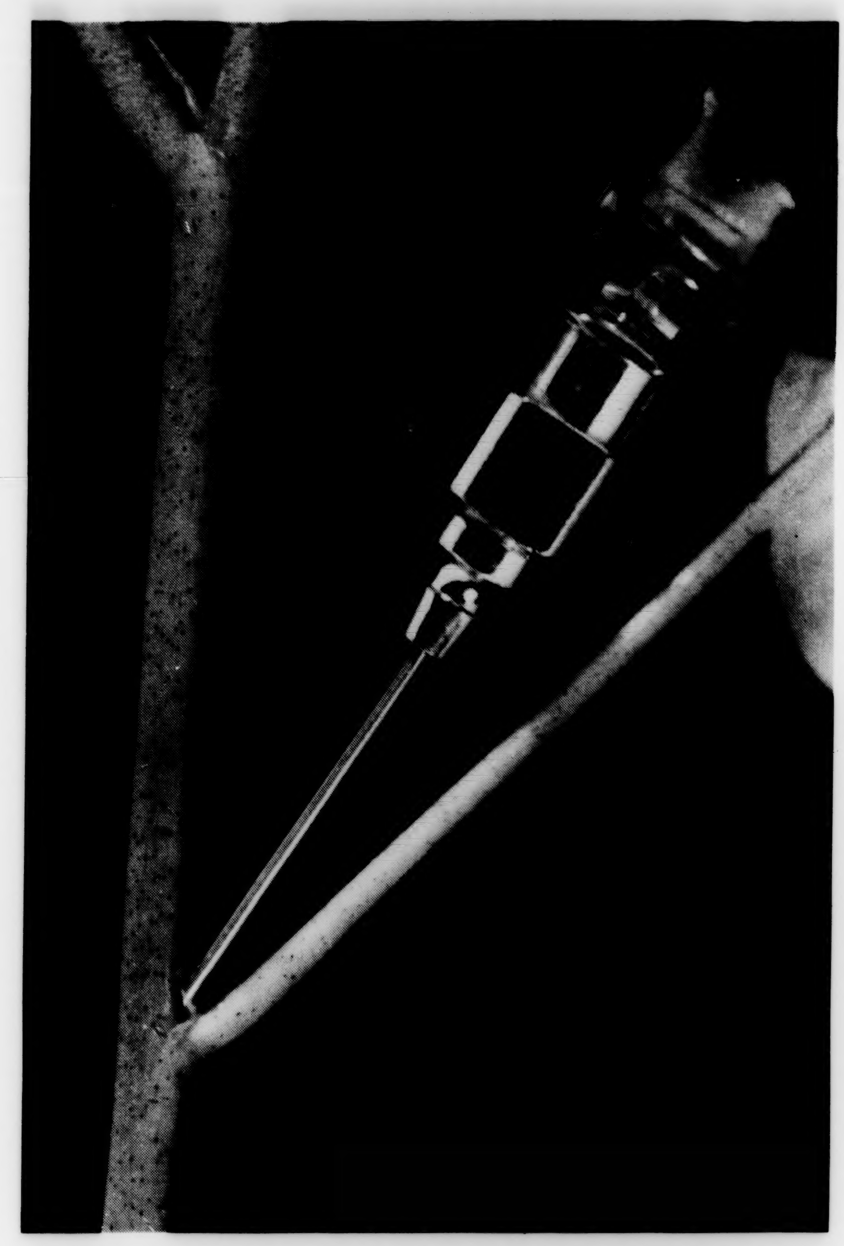


Fig. 2 Inoculation of a cotton plant by puncturing the stem with a 22 g hypodermic needle. Note the droplet of spores of *Verticillium albo-atrum* in the axil. This method has also been effective for inoculation of plants in field experiments

all the plants were defoliated 14 days after inoculation. At 5×10^4/ml defoliation had just begun but at 5×10^3/ml and $0{\cdot}5 \times 10^2$/ml, no symptoms were seen until 5 days later (Fig. 4). Ultimately all plants eventually had symptoms of Verticillium wilt. This and other experiments indicated that a concentration of 10^5 spores/ml was high enough to cause a maximum expression of Verticillium wilt in 5–10 days. As a safety factor we now use 10^6 spores/ml as inoculum.

As an example of the use of the stem inoculation method for testing a systemic chemical, Benlate was applied to roots of 1 or 2 month-old plants as a drench in sand, sufficient to give a concentration of 10 and 50 ppm 10 days prior to inoculation. Nearly complete control of Verticillium wilt was obtained.[13] The results of another experiment in which young (3-week-old) plants were treated with Benlate and TBZ at 10, 20, and 40 mg per pot (800 g sand) are shown in Fig. 5. Plants were completely protected by Benlate and TBZ when applied before inoculation of stems. TBZ was more phytotoxic than Benlate especially on young plants. In other similar experiments TBZ applied to 1·5 month old plants prevented Verticillium wilt at a concentration of 50 mg per pot (Fig. 6). Interveinal necrosis caused by TBZ (plant D) can be seen. These experiments demonstrated presumptively that both Benlate and TBZ had the capacity to prevent and/or delay disease. Numerous experiments in which the inoculum concentration was increased to 10^7/ml indicated that the controlling effect of Benlate was not diminished by inoculum concentration.

Double pot method Another method to achieve the separation of inoculum from fungicide is the 'double pot method'. The plant was grown in a square plastic pot, and when 2–3 weeks of age, the intact root ball was changed to a pot from which the bottom has been removed. The potted plant was then set on the top of another pot which contained soil or sand which had been mixed with the chemical (Fig. 7). After the roots had grown into the treated soil for 7–15 days and had an opportunity to take up the chemical, the roots in the upper pot were inoculated by pouring a suspension of spores over the roots on the periphery of the root ball. This was done by squeezing the sides of the square polypropylene pot and pouring the suspension of spores (50 ml adjusted to 10^6/ml) over the roots on each of the four sides of the root ball. The plants treated in this way are not physiologically injured.

By this method control or delay of symptom expression by the chemical can be presumptively attributed to a systemic effect which is not due to the direct contact effect of the fungicide. For chemicals that are volatile or water soluble, this method would be less advantageous than for the non-volatile and relatively insoluble compounds, TBZ and Benlate.

As an example of this method, TBZ was incorporated in the soil in the lower pot at 100 ppm and 10 days later the roots of the plants in the upper pot were inoculated with a suspension of spores (Fig. 8). TBZ in the lower pot, where there was no inoculum, prevented symptom expression.[15]

The effect of organic material in soil was studied using the double pot method. One set of these pots contained a mixture of 50 per cent peat moss and 50 per cent sand

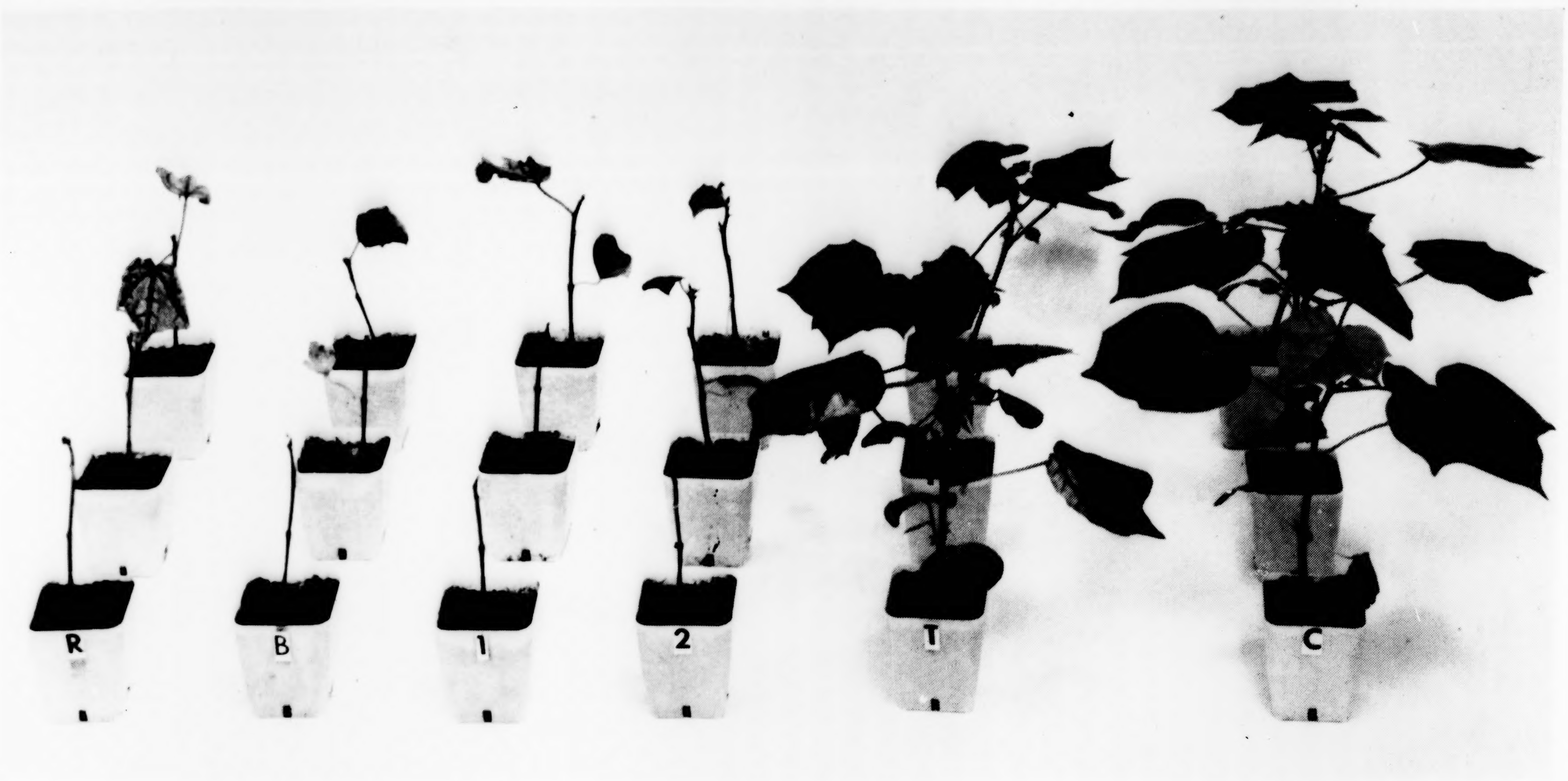


Fig. 3 Effect of needle puncture inoculation of cotton plants 3 weeks old with spores (10^6/ml) of *Verticillium albo-atrum* (V3H) at different positions on the plant. R, upper tap root; B, base of stem; 1, first node; 2, second node; T, growing tip; and C, control, stems at first node were punctured with a sterile needle

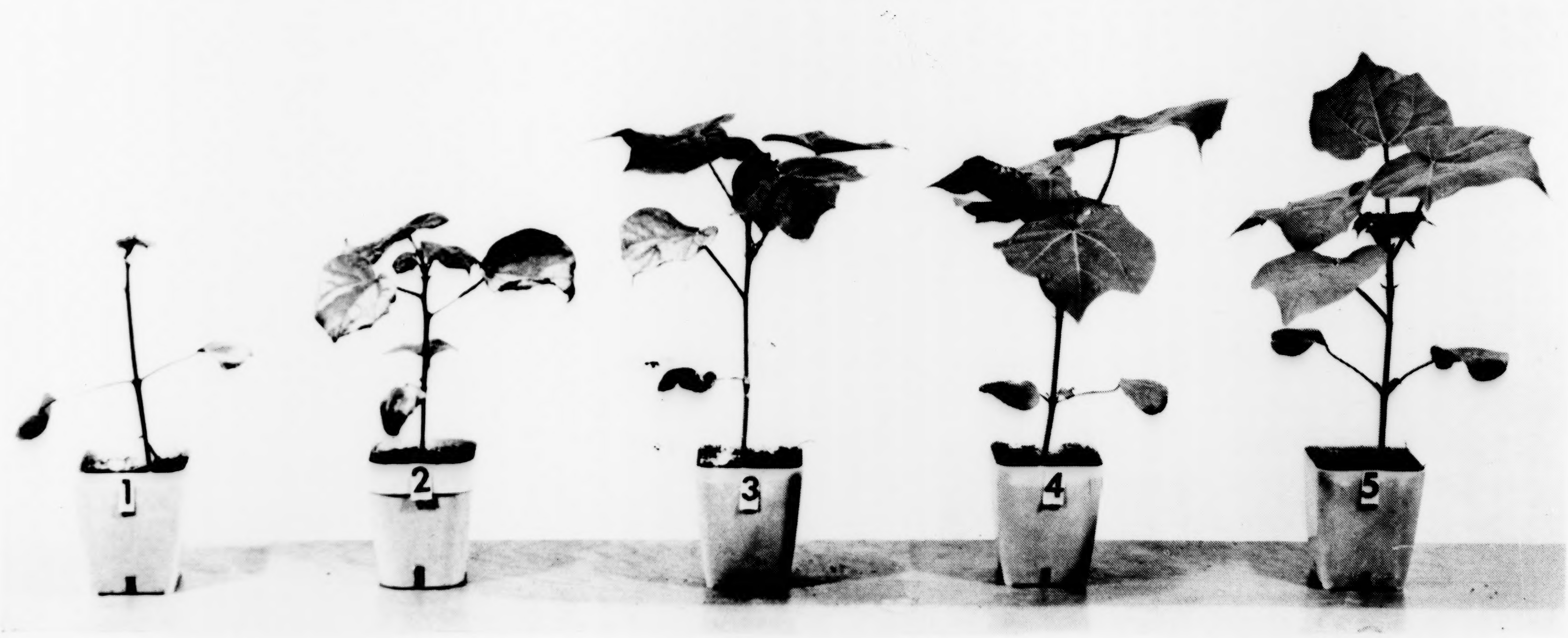


Fig. 4 The effect of stem inoculation of cotton plants 3 weeks old with different concentrations of spores of *Verticillium albo-atrum* (V3H). Pot 1, 5×10^5 spores/ml; pot 2, 5×10^4/ml; pot 3, 5×10^3; pot 4, $0{\cdot}5 \times 10^1$; and pot 5 control, not inoculated

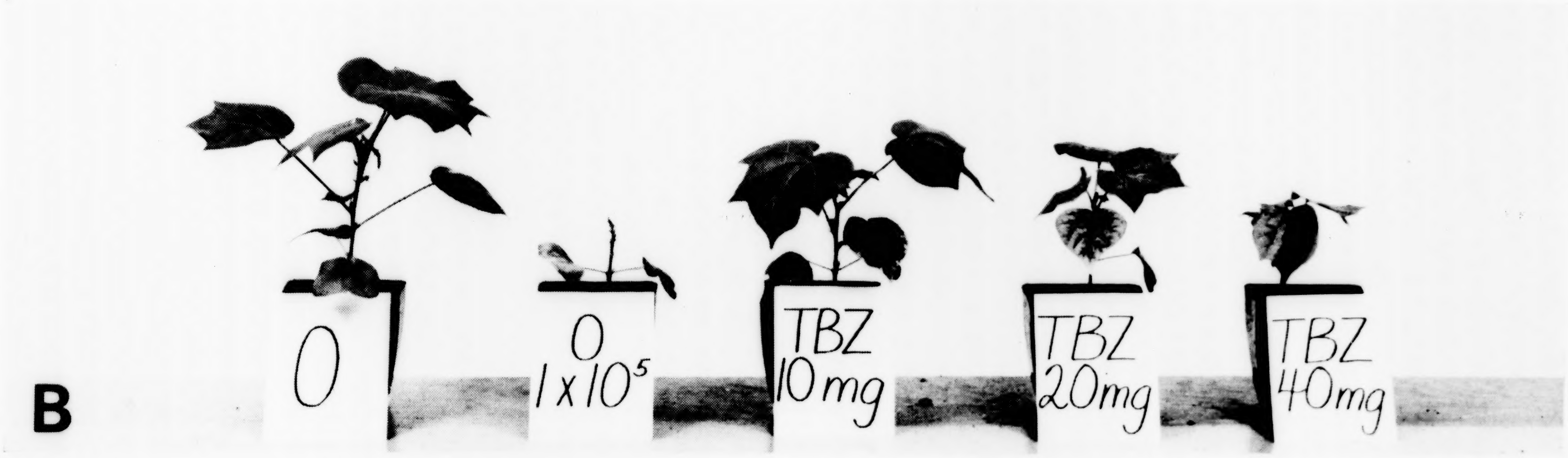


Fig. 5 Three week old plants of cotton, growing in sand in which (A) Benlate (1991) and (B) TBZ had been incorporated at different concentrations prior to planting, were stem inoculated at 3 weeks of age with *Verticillium albo-atrum* (V3H). Plant at the left (0) received no chemical and was not inoculated. Plant labeled 0, 1 × 10^5 received no chemicals and was inoculated with V3H spores (1 × 10^5/ml). All other plants were inoculated. All dosages of the chemical controlled the disease; however TBZ was more phytotoxic than Benlate (1991)

Table 1
The effect of peat moss in sand on the activity of Benlate against *Verticillium albo-atrum* on plants inoculated by stem puncture

	Plants showing symptoms of Verticillium wilt							
	Soil used in lower pot							
	Peat–Moss–Sand				Sand			
Dosage in soil in lower pot (mg)	Days after inoc. 16	Days after inoc. 25	Xylem discoloration[a]	Reisolation from 7th node Per cent	Days after inoc. 16	Days after inoc. 25	Xylem discoloration[a]	Reisolation from 7th node Per cent
0	8/8	8/8	2·9	—	0/8	7/8	2·9	—
7·5	4/8	6/8[b]	3·1	75	2/8	6/8[b]	2·9	75
37·5	4/8	8/8	2·9	100	2/8	1/8	3·0	

[a]Xylem discoloration at the first node was rated 0–5, where 0 = none and 5 = complete discoloration
[b]At 7·5 mg/pot in peat–moss–sand all 6/8 affected plants were defoliated; but in sand, although 6/8 plants had chlorotic symptoms on leaves, there was no defoliation

Fig. 6 Effect of TBZ, drenched on sand, on Verticillium wilt following inoculation by stem puncture with *Verticillium albo-atrum* (V3H). Plants were 6 weeks old when inoculated. A. control, non-treated, non-inoculated; B. non-treated, inoculated; C. treated by drenching 50 mg TBZ/pot on sand (800 g) and inoculated; and D. treated with TBZ as in C but non-inoculated. Interveinal leaf necrosis is due to phytotoxicity of TBZ

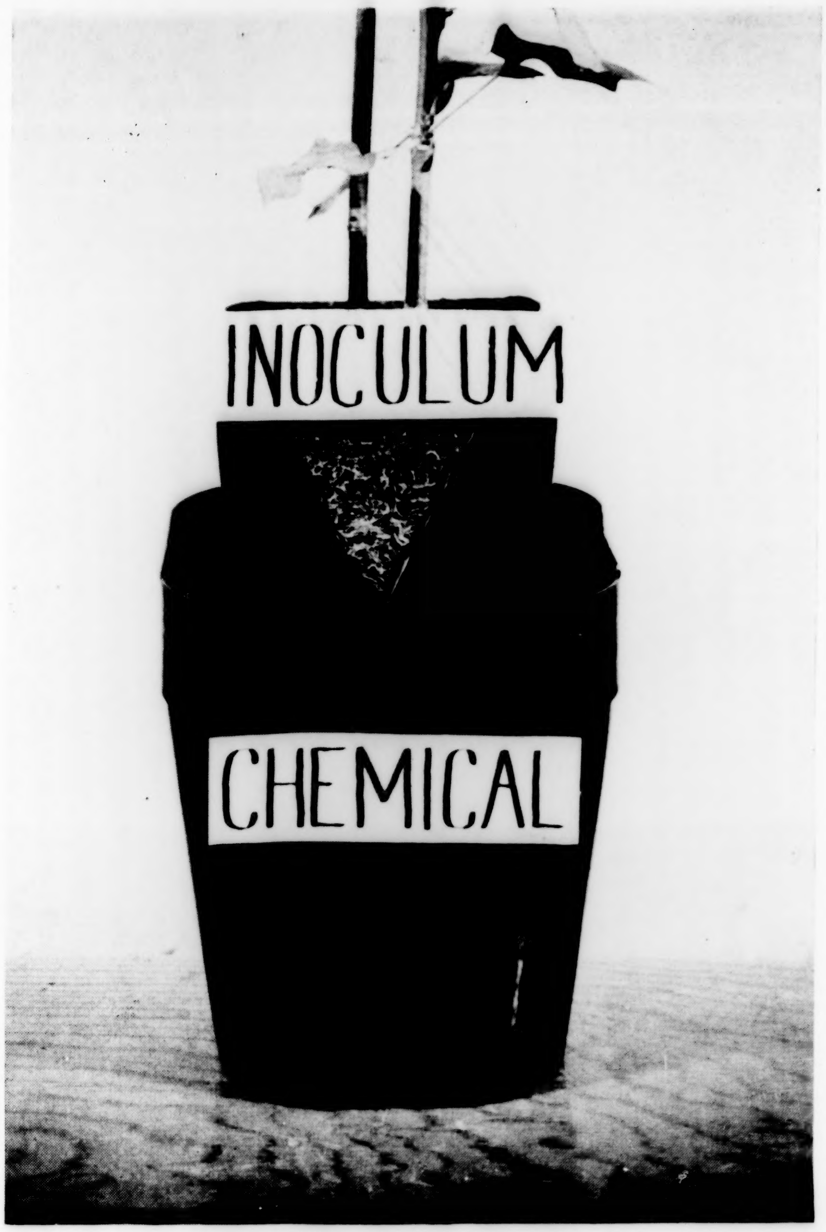
(PS = UC Mix)[1] and another set contained sand. Benlate was incorporated with each of the substrates in the lower pot at concentrations of 7·5 and 37·5 mg/pot. These concentrations are approximately 15 and 75 ppm respectively based on dry weight of the lighter PS. Control pots contained only PS or sand. Roots from one-month-old plants in bottomless pots were allowed to grow into the lower pots containing Benlate for 10 days. Plants were then inoculated by puncturing the stems.

On the 25th day after inoculation all of the eight inoculated plants in nontreated sand or PS had wilt symptoms, but in sand treated with Benlate at 7·5 and 37·5 mg/pot there was considerable reduction in disease. However, in the PS pots Benlate at either concentration did not control the disease (Table 1) (Fig. 9).

Since it appeared most likely that peat moss adsorbed Benlate, steamed sand has been used for all subsequent primary screening experiments.

Other Methods Sinclair and his students developed several methods[6] for study of systemic fungicidal control of the seedling disease of cotton caused by *Rhizoctonia solani* Kühn. A clay pot was filled about $\frac{3}{4}$ full with Vermiculite which contained the fungicide. The Vermiculite was covered with a polyethylene film. Cotton seeds were planted on small holes punched in the film and covered with Vermiculite. After the seedlings emerged, the Vermiculite above the sheet of polyethylene was infested with *Rhizoctonia*. A reduction in numbers and severity of lesions on hypocotyls, which had not been in contact with the chemical, was interpreted as evidence for translocation.

Fig. 7 Double pot method. The plant is either grown in a 'bottomless' polypropylene pot or placed in one prior to being set on another pot containing the chemical. The chemical can either be incorporated in the soil or be drenched on the exposed soil. The side of the upper pot was cut away to show the prolific distribution of white roots which may be effectively inoculated with spores after exposing them by squeezing the side of the pliable polypropylene pot. Placement of spore inoculum directly on top of soil is much less effective most likely due to the lower numbers of roots on the soil surface and the relatively short life of *Verticillium* spores



They also described a technique utilizing a wide mouth Erlenmeyer flask containing the chemical mixed in Vermiculite. Seeds were planted in a cup formed in a sheet of plastic film over the mouth of the flask. Seeds were planted in Vermiculite in the cup. After roots had grown downward through holes in the plastic and contacted the fungicides in the flask, the hypocotyls were inoculated with agar discs of *Rhizoctonia solani*. A reduction in numbers and size of lesions indicated that the oxathiin chemicals, 'Plantvax' and 'Vitavax', had translocated.

Determination of preventive, delaying or therapeutic effects

After presumptive evidence for a systemic effect of a chemical on disease has been obtained, it is necessary to know if the chemical prevents, delays or cures the disease. By recording the onset of disease daily, a rate of disease expression can be obtained. The data in Table 2 show the effect of increasing the concentration of Benlate in soil on the incidence of disease caused by the severe (V3H) and mild (V22) isolates of *V. albo-atrum*. At a dosage of 50–100 ppm symptom expression was prevented and at 25 ppm it was delayed.

To establish the degree of prevention it was important to reisolate *V. albo-atrum* from treated and inoculated plants in an attempt to determine whether or not the fungus had been eradicated. At 50 and 100 ppm the fungus was not recovered but at 25 ppm Verticillium was recovered in 30 per cent of the stems.

A successful systemic fungicide should be capable of a

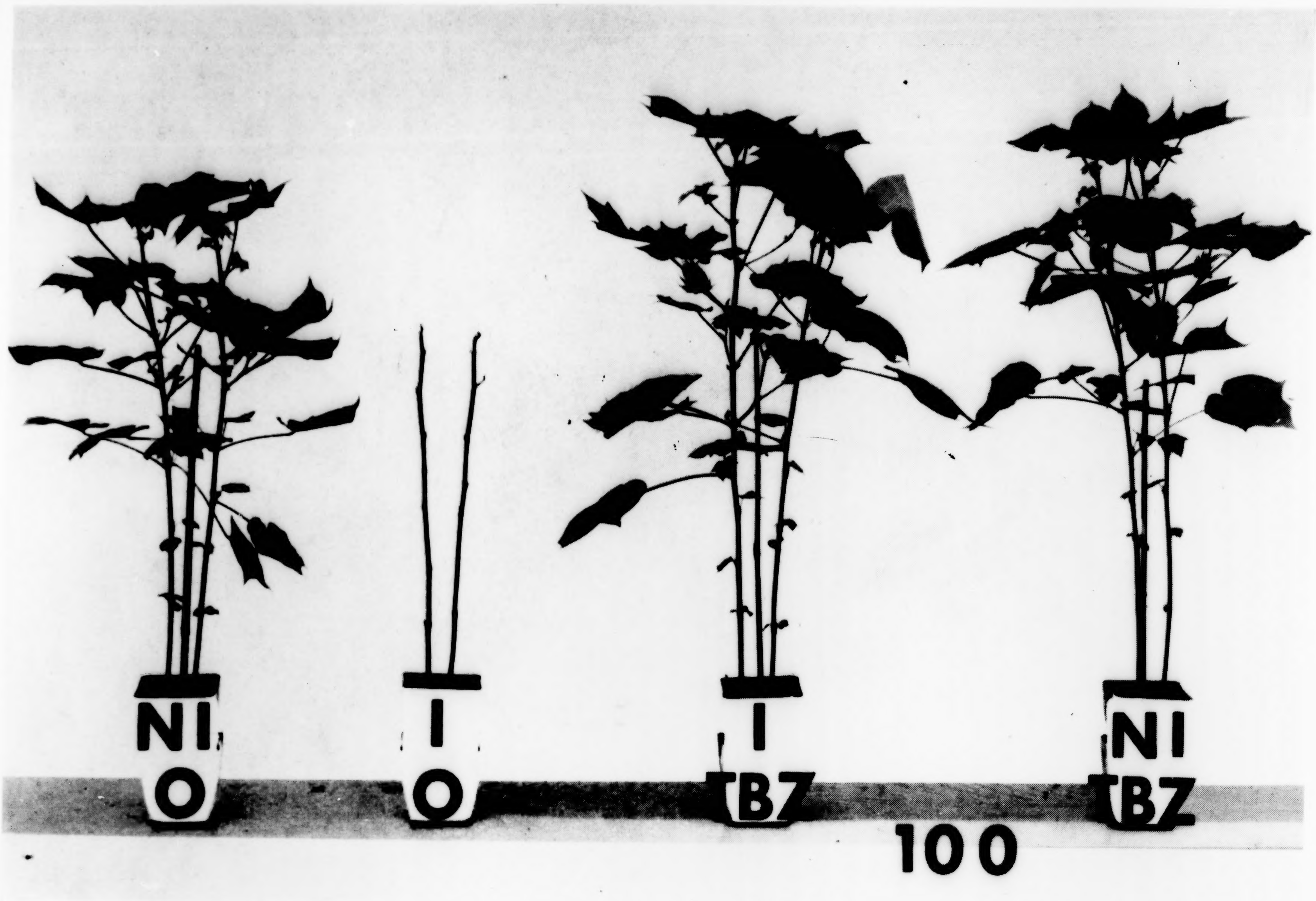


Fig. 8 Effect of incorporating TBZ in soil (100 ppm, w/w) in the lower pot prior to inoculating roots of plants in the upper pot with 100 ml of a suspension of spores (5×10^6/ml) of *Verticillium albo-atrum* per pot

curative as well as a preventive effect. A therapeutic effect can be determined by treating the roots of the plant at periodic intervals before and after inoculation. By adjusting the time of treatment before and after inoculation the possibility of therapeutic activity can be determined.

The results of the experiment shown in Fig. 10 in which roots of plants were inoculated before and after drenching the soil with Benlate at 50 mg per pot of sand (800 g) indicate that Benlate completely prevented symptom expression by the V3H isolate. The absence of xylem discoloration does not necessarily prove that the fungus was absent at the first node but strongly indicates this.

In this experiment the inoculum was applied to the same roots which were treated by drenching with Benlate before and after inoculation. Thus we would expect control by any fungicide by treatment of soil prior to inoculation, but if control is obtained by treatment five days after inoculation, it indicates that Benlate must penetrate the inoculated roots. Previous studies indicated that infection of roots occurred in less than three days.

In Fig. 11 another approach to the demonstration of a curative effect is shown. Benlate (BBC) was applied to plants growing in sand (800 g/pot) by drenching at minimal dosages (5 mg/pot). The effect of these concentrations

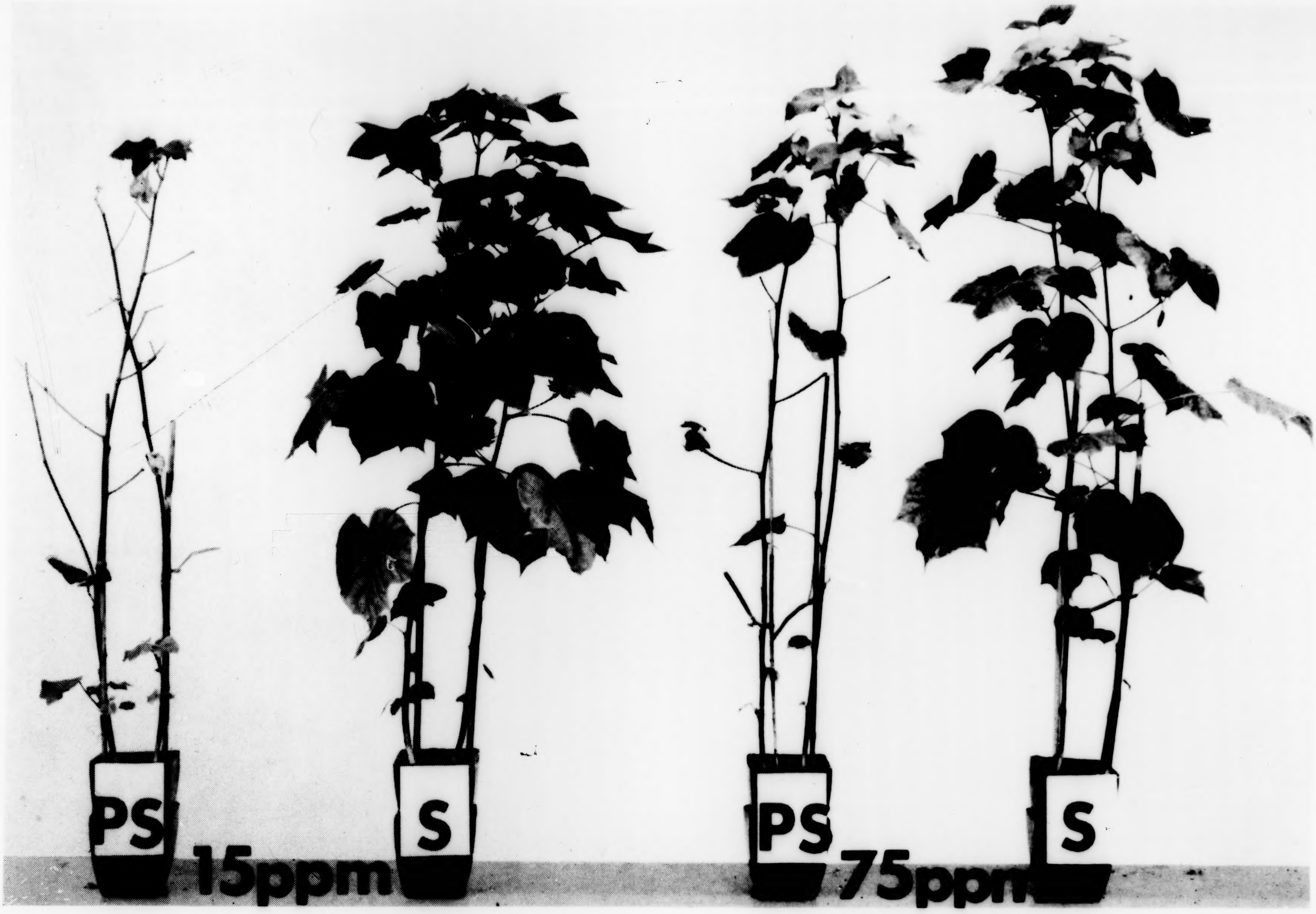


Fig. 9 Effect of mixing Benlate (BBC) with a 50 per cent peat moss–50 per cent sand mixture and with sand in the bottom pot on the development of Verticillium wilt following inoculation by stem puncture. The 15 (7·5 mg/pot) and 75 ppm (37·5 mg/pot) concentrations of Benlate in the bottom pot are calculated on the basis of the weight of the lighter PS mixture. The non-treated inoculated control plant (not shown) was similar to the plants in the PS pot at 15 ppm

of Benlate on two different strains of *Verticillium* (v3H defoliates plants, v22 does not) inoculated into stems are also shown. When Benlate was drenched on soil with 3 to 5 mg dosages before inoculation, nearly complete control of both isolates was obtained. When the number of treatments with Benlate was decreased to one dosage (5 mg) 1 day before inoculation, v3H (severe) was not controlled but v22 (mild) was controlled. Likewise as the numbers of 5 mg treatments after inoculation increased, the degree of control also increased. However, the degree of control of the v22 mild isolate was much greater than for the severe v3H.

These data and those in Table 2 indicated that the virulence of the isolate is extremely important for testing systemic fungicides.

Further experiments (to be reported elsewhere) have been conducted using the technique of inoculation at different time intervals after treatment. These indicated that Benlate has a therapeutic effect and that the severe v3H isolate is much more difficult to suppress by a systemic fungicide than the milder v22.

It is of theoretical as well as practical interest to speculate on the problem of controlling a systemic fungus with a systemic fungicide.[15] Plugging of xylem tissue, one of

Table 2 Effect of several concentrations of Benlate in soil on the incidence of Verticillium wilt caused by a defoliating (V3H) and nondefoliating (V22) strain of *Verticillium albo-atrum* following inoculation by stem puncture with spores (10^6/ml)

Benlate in soil ppm	Inoculant	Symptoms[a] Days after inoculation 0	7	18	29	Xylem discoloration[b]	Reisolation from stem (7th node)
							Per cent
0	0	0	0	0	0	0·0	0
0	V3H	0	10EC	5D, 5EC	10D	4·0	100
0	V22	0	10EC	10EC	10EC	4·4	30
25	V3H	0	0	5EC	10EC	0·7	30
	V22	0	0	2EC	1EC	0·1	0
50	V3H	0	0	0	0	0·0	0
	V22	0	0	0	0	0·1	0
100	V3H	0	0	0	0	0·0	0
	V22	0	0	0	0	0·0	0

[a] The no. of 10 plants which had following symptoms: E = epinasty; C = chlorotic spots on leaves; and D = defoliation

[b] Xylem discoloration at the first node was rated 0–5 where 0 = none and 5 = complete discoloration

BBC mg/pot	Root Drench Inoculated	Results Condition	Xylem Disc.
50	3 days before	Healthy plants	0
50	1 day before	Healthy plants	0
0	0 day before	Defoliated plants	4·0
50	1 day after	Healthy plants	0
50	3 days after	Healthy plants	0
50	5 days after	Healthy plants	0

Fig. 10 The effect on disease expression of Benlate used as a drench in 4-inch pots of sand (800 g) containing one-month old plants before and after inoculation by root drench with spores of *Verticillium albo-atrum* (V3H). Xylem discoloration at the first node was graded using the criteria: 0 = none and 1–5 increasing intensity of discoloration

the physiological effects of a vascular fungus,[9] could effectively prevent the upward movement of systemic chemicals or at least limit their activity to newly formed xylem tissue. If this is true a systemic fungicide might be needed prior to or shortly after infection before formation of vascular occlusions. When we applied Benlate later than 3 to 5 days after inoculation with the severe V3H isolate we were unable to control the onset of Verticillium wilt.

The detection of chemicals in treated plants

Bioassay

The most reliable proof of translocation is evidence of the existence of the chemical or its metabolized products at a point remote from the place of application.

Some toxic diffusible chemicals can be rapidly detected by bioassay in plant tissue by placing the tissue in a petri dish of agar seeded with fungus spores. Munnecke[22] reported use of such a technique for assay of the presence of chemicals in soil. Obviously fungitoxic chemicals are more easily detected by bioassay than those that are nontoxic.

Helton and Harvey reported detection of cycloheximide and several other systemic compounds in wood chips (bark and xylem tissue) from branches of prune trees (*Prunus domestica* L.) which had been treated by trunk injection. They plated the chips on plates of malt agar inoculated in the center with *Cytospora cincta* Fr.[17]

Although a bioassay method does not yield as conclusive proof as chemical methods for presence of a toxic substance, it is a valuable tool in that it is often more sensitive than a chemical method and requires less time. In all cases a bioassay should be correlated with a chemical method if possible. Sun[29] discussed the principles of bioassay of pesticide residues from the standpoint of insecticidal compounds.

We described a bioassay method to detect a toxic substance in leaves of cotton plants treated with Benlate.[13] Since *Penicillium expansum* Lk. ex Thom. was about four times as sensitive (0·1 μg/ml) as *V. albo-atrum* to Benlate in an *in vitro* test, this fungus was used in a bioassay. Spores of *P. expansum* were incorporated in cooled but fluid Sabouraud's agar (25 ml/85 mm petri dish). Discs of treated and untreated leaves were incubated on the solidified agar for 40 hrs. Clear zones of inhibition were associated with leaf discs from treated but not from untreated leaves (Fig. 12).

To utilize the casual fungus, *V. albo-atrum*, in the bioassay, we developed techniques for its use. Since spores of *V. albo-atrum* were much more sensitive than those of *P. expansum* to the temperatures (42–45° C.), approximating those of cooled but still fluid agar, plates were seeded by spraying the plate with a suspension of washed spores rather than incorporating them in agar.

Treatment								Disease (31 days)			
BBC mg/pot	Days							Per cent		Xylem Discoloration	
				Stem Inoc.				V - 3H (severe)	V - 22 (mild)	V - 3H (severe)	V - 22 (mild)
	−5	−3	−1	0	+1	+3	+5				
15	■	■	■					0	0	1·0	·0
10		■	■					12	0	2·4	1·6
5			■					100	12	3·3	2·5
0								100	75	3·1	2·6
5					■			75	25	3·5	3·1
10					■	■		25	0	1·9	0·8
15					■	■	■	0	0	1·0	0·9

Fig. 11 The effect of drenching sand with 5 mg/pot dosages of Benlate (BBC) at different times before and after inoculation by stem puncture on disease expression. The V3H isolate defoliated plants but V22 did not. Xylem discoloration at the first node was graded using the criteria: 0 = none, and 1—5 indicating increasing intensity of discoloration

Results of subsequent experiments indicated that the size of the zone of inhibition (indicative of the sensitivity of the test fungus) could be increased by allowing the substances in the disc to diffuse in agar for 24 hours prior to seeding the plate with the fungus and by reducing the amount of agar in the petri dish. For instance 1 μg of TBZ was placed on 12·7 mm filter paper discs on some plates containing 10 ml and on others 15 ml of potato dextrose agar (PDA). After 24 hours the agar was sprayed with spores. Two days later the area of the zone of inhibition on plates containing 10 ml of agar was 1,142 mm^2 compared to 708 mm^2 on plates containing 15 ml.

The sensitivity of detection of a chemical in plant tissue could also be increased by freezing the plant tissue prior to placing on agar. For instance stems of plants (1-month-old) which had received 50 mg of TBZ in sand by drenching 1 week prior were surface sterilized in a proprietary sodium hypochlorite solution (0·52 per cent) for 5 minutes. Some of the stem sections were frozen at −18 C. for 1 day and others were not. Stem pieces were plated on PDA amended with streptomycin (30 ppm) to suppress bacterial contamination. The size of the zone around the frozen sections was much greater than around the unfrozen (Fig. 13).

One of the difficulties with the diffusion bioassay method is determination of quantity of a toxic substance in plant tissue. Since all of the toxic substance in plant tissue does not diffuse out, the relative value indicated by the area of the zone of inhibition is probably lower than the amount of chemical actually in tissue. When leaf or stem tissue is extracted and the extract bioassayed, the amount of chemical in this tissue can be more quantitatively measured. This method was used by Dekhuijzen.[7]

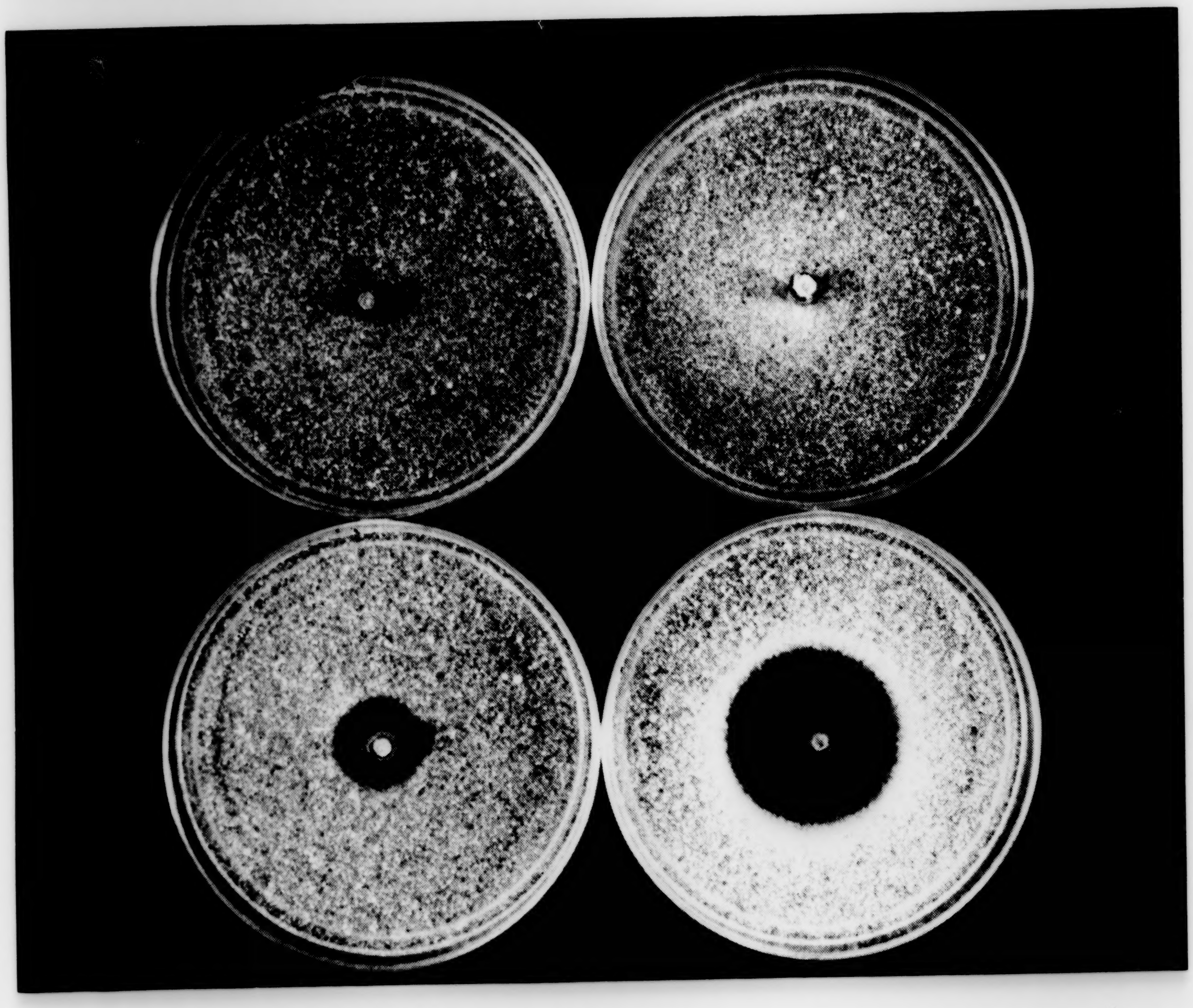
The *in vitro* assay of the toxicity of TBZ and Benlate* by the agar diffusion method does, however, appear to be quantitative. There is a proportional increase in the size of the zone of inhibition with increase in quantity of the fungicide (Fig. 14). The least quantity detectable by a zone of inhibition was 0·3 μg of TBZ and 0·4 μg of Benlate. These differences may not be related as much to differences in toxicity of the chemical as to solubility and diffusibility. Although these *in vitro* data are by no means definitive, they indicate that both Benlate and TBZ are fungitoxic at low concentrations and that either the parent compounds or their toxic breakdown products exist in plant tissue at a point beyond the place of application.

Detection of fungitoxic chemicals by chemical methods To prove that a chemical has been translocated, chemical methods must be employed. Wain and Carter[12] pointed out that this has seldom been done for systemic fungicides. Although the bioassay method is valuable in that it quickly indicates whether or not there is evidence for translocation, ultimately chemical methods must be employed.

*Both technical grade chemicals from the manufacturers.

Fig. 12 Zone of inhibition of the fungus *Penicillium expansum* around a disc of cotton leaf tissue from a plant that received 50 mg/pot (800 g) of Benlate. Disc at right was from a nontreated plant

Fig. 13 Effect of freezing cotton stem tissue at −18 C for 24 hrs on diffusion of TBZ. Top row: stem sections from nontreated plants. Bottom row: stem sections from plants treated with TBZ at 50 mg/pot. Sections on the left were not frozen but sections at right were



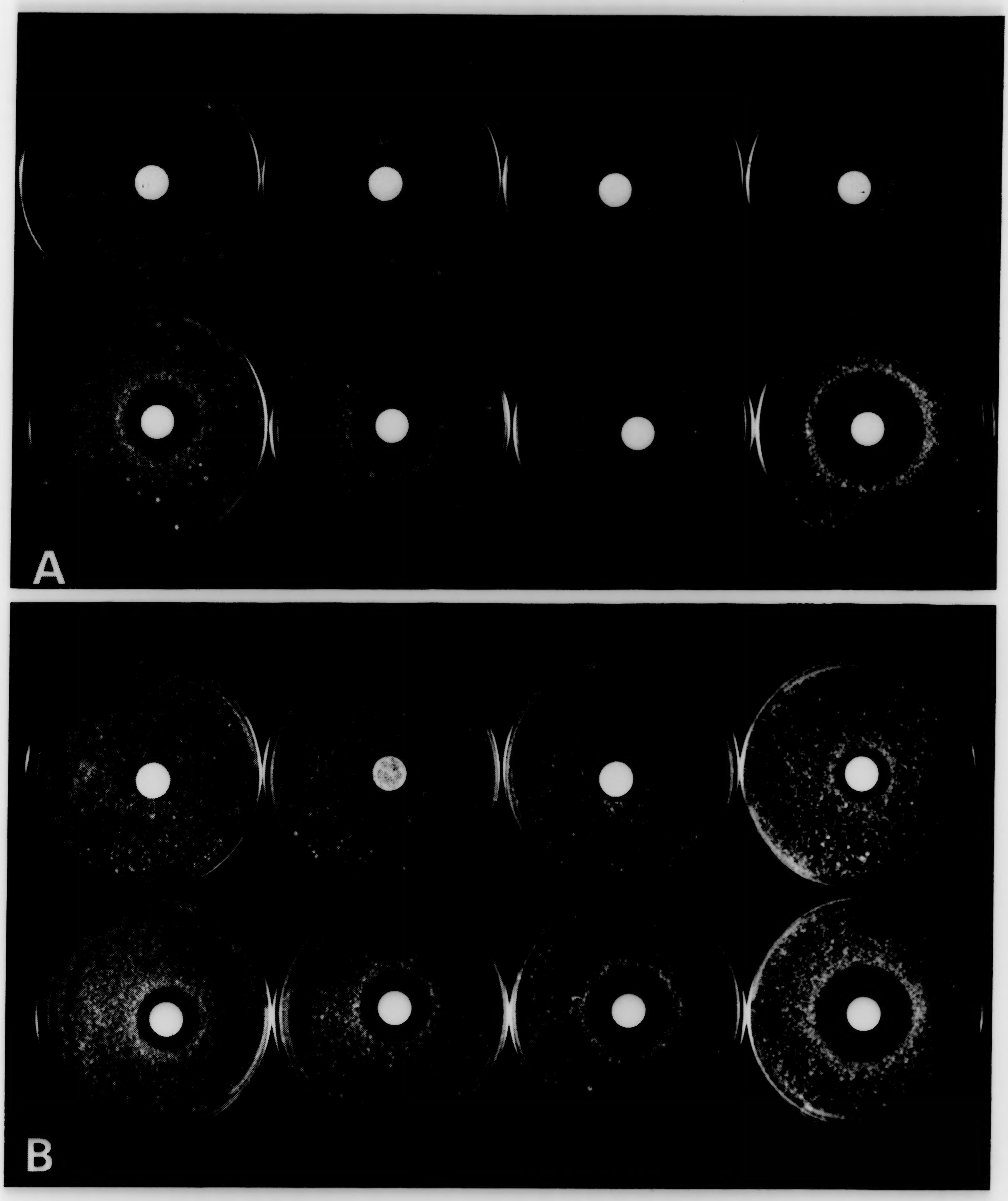


Fig. 14 Comparison of the zone of inhibition on a plate of potato dextrose agar (10 ml) seeded with *Verticillium albo-atrum* on which cellulose discs containing TBZ or Benlate were placed.

A TBZ – top row 0, 0·2, 0·3, 0·4 and bottom row 0·5, 0·6, 0·8 and 1·0 μg/disc

B Benlate concentrations were the same as A

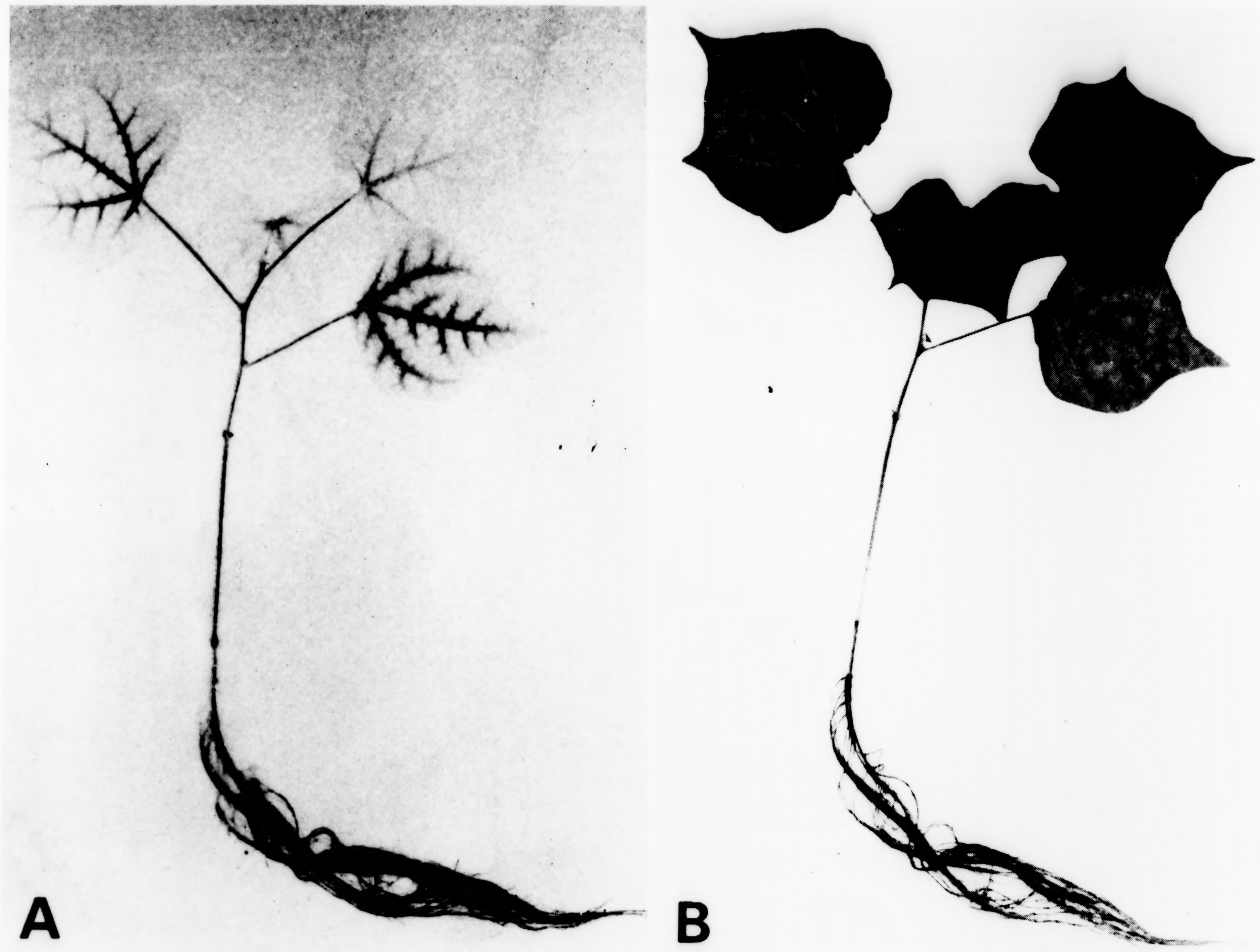


Fig. 15 A Radioautograph of a cotton plant grown 5 days in a nutrient solution containing 0·3 μC of TBZ–C^{14}
B Photograph of the dried plant in A

Perhaps one of the most elegant papers, which deals with metabolism of sodium dimethyldithiocarbamate (NaDDC) in plant tissue, is that of Dekhuijzen[7] who developed the paper chromatographic method of detection of fungitoxic chemicals reported by Weltzein.[33] NaDDC and the metabolic products of it were detected in plants by spotting plant sap on Whatman No. 3 filter paper and running descending propanol-water, 85:1, over the spot for 16 hours. When the strips of paper were dried at 30°C. and sprayed with spores of *Glomerella cingulata* in a nutrient solution, the spots containing a fungitoxic compound on the paper were detected by the absence of mycelial growth on the spot which contained a fungitoxic chemical. This useful technique might be termed 'development' by a fungus.

Another method used by Dekhuijzen[7] consisted of dipping a 0·9 cm paper disc in plant sap prepared from a treated plant. The disc was dried at 30°C., sterilized in chloroform vapor and incubated on a plate of agar seeded with *Glomerella cingulata*. A zone of inhibition of the fungus indicated the presence of a fungitoxic substance.

Treatment of plants with labeled compounds and detection of the labeled compounds beyond the point of application by liquid scintillation and radioautography is

extremely sensitive. That the labeled compound is indeed the originally applied molecule or a product of it requires further proof, such as the isotope dilution technique.[24]

A cotton plant treated in nutrient solution with ^{14}C-TBZ (0·3 μC) is shown in Fig. 15. The radioautograph indicated that the concentration of the labeled compound decreased with increasing height above the roots. These observable data were correlated with counts of radioactivity in stem and root tissue by liquid scintillation and spectrometry (to be reported elsewhere by M-C. Wang, J. J. Sims and D. C. Erwin).

Fielding and Rhodes[16] showed by radioautography that ^{14}C-chloroneb moved upward from roots to the hypocotyl area but not to the upper part of the plant. This correlated well with the effect of the translocated chemical on disease expression caused by *Rhizoctonia solani* when inoculated on hypocotyls of plants treated in the root zone.

We now have spectrophotofluorometric evidence obtained by Merck Chemical Division, Rahway, New Jersey, for the presence of TBZ in leaves and stems of plants treated with TBZ in the root zone. The details of this work will also appear elsewhere.

Discussion

The methods suggested here give presumptive evidence that a compound is systemic based on the principle that the chemical must be translocated to a position remote from the point of application of the chemical. Further confirmation can be obtained by bioassays of treated plant material for fungitoxic substances, by radioautography, and by chromatographic means. Ultimately, definitive chemical analyses must be used to establish the identity of the translocated chemical in plant tissue. Wain and Carter[32] indicate that this has seldom been done with fungicides. A notable exception is the work by Crowdy *et al.*[5] who reported isolation of pure griseofulvin and chloramphenicol from the shoots of root-treated broad bean plants.

In screening systemic chemicals against a vascular parasite the method of inoculation of plants is perhaps more critical than with leaf parasites due to the many uncontrollable factors in the soil where infection initially takes place. Many people rely on nonsterilized soil because it is more natural. Other soil pathogens however, many of which are not affected by certain chemicals, could cause disease and thus negate the effects that the chemical might exert against a particular pathogen. For instance neither TBZ nor Benlate is effective against *Pythium*. It seems most logical that for primary screening a light soil or sand which has a minimal adsorptive capacity and which is freed of plant pathogens would be the best choice. Baker's[1] recommendation of heating soil to 82 C. for 0·5 hour with steam to rid soil of all known plant pathogens has been effective in our work.

It has also been our experience that expression of disease symptoms by plants growing in either naturally or artificially infested soil is much slower than with the root or stem inoculation technique. For primary screening this would be a decided disadvantage. However for advanced testing, utilization of both artificially and naturally infested soil is necessary.

It is shown clearly in our work that strains of *Verticillium albo-atrum* vary in their virulence toward cotton. Selection of the strain that produces the level of disease expression desired is, therefore, imperative. One cannot argue that selection of the most severe strain of this fungus is always the most logical approach. We selected the V3H isolate, not entirely because it was the most severe, but also because it represented the typical defoliating isolate found in nature. If the milder type (exemplified by V22) was the usual, natural strain, it might be the most logical choice.

The study of systemic fungicides is relatively new and offers many challenges which require new research approaches. We need to know more about how the newer chemicals are taken up by plants. Is the process physiologically active or passive? What is the efficiency of uptake? Is the fate of the chemicals in all plants the same? These are but a few of the questions that can be asked and must be answered if systemic fungicides are to take their place in the practical control of plant diseases.

References

1 Baker, K. F. (ed). 1957. University of California, Agr. Extension Service Manual 23, 332 pp.

2 Brinkerhoff, L. A. 1949. *Phytopathology*, **39**, 495.

3 Bugbee, W. M. and Presley, J. T. 1967. *Phytopathology*, **57**, 1264.

4 Carns, H. R., Presley, J. T., Schnathorst, W. C., Harrison, G. K. and Taylor, F. E. 1964. Proc. 24th meeting, National Cotton Disease Council Jan. 7–8, 1964, p. 83–89.

5 Crowdy, S. H., Gardner, D., Grove, J. F. and Pramer, D. 1955. *J. exp. Bot.* **6**, 371–383.

6 Darrag, I. E. A. and Sinclair, J. B. 1968. *Plant Disease Reporter*, **52**, 399–403.

7 Dekhuijzen, H. M. 1964. *Plant Pathology, Netherlands*, **70**, Suppl. 1, 1–75.

8 Delp, C. J. and Klopping, H. L. 1968. *Plant Disease Reporter*, **52**, 95–99.

9 Dimond, A. E. 1967. *In* Mirocha, C. J. and Uritani, I. *ed.* '*The dynamic role of molecular constituents in plant–parasite interaction*'. Bruce Publ. Co., Minneapolis, pp. 100–120.

10 Dimond, A. E., Davis, D., Chapman, R. A. and Stoddard, E. M. 1952. *Conn. Agr. Exp. Sta. Bull.* **557**, 82 pp.

11 Erwin, D. C. 1968. Proc. First International Congress of Plant Pathology, London, 55.

12 Erwin, D. C. and DeWolfe, T. A. 1968. *Plant Disease Reporter*, **52**, 281–283.

13 Erwin, D. C., Mee, H. and Sims, J. J. 1968. *Phytopathology*, **58**, 528–529.

14 Erwin, D. C., Moje, W. and Malca, I. 1965. *Phytopathology*, **55**, 663–665.

15 Erwin, D. C., Sims, J. J. and Partridge, J. B. 1968. *Phytopathology*, **58**, 860–865.

16 Fielding, Max and Rhodes, R. C. 1967. Proc. National Cotton Council, Beltwide Cotton Production and Mechanization Conf. Jan. 9–13, 1967.

17 Helton, A. W. and Harvey, A. E. 1963. *Phytopathology*, **53**, 895–898.

18 Kendrick, J. B. Jr. and Zentmyer, G. A. 1957. *Adv. Pest Control Res.* **1**, 219–275.

19 Keyworth, W. G. and Dimond, A. E. 1952. *Phytopathology*, **42**, 311–315.

20 Malca, I., Erwin, D. C., Moje, W. and Jones, Barbara. 1966. *Phytopathology*, **56**, 401–406.

21 Mathre, D. E., Erwin, D. C., Paulus, A. O. and Ravenscroft, A. V. 1966. *Plant Disease Reporter*, **50**, 930–933.

22 Munnecke, D. E. 1958. *Phytopathology*, **48**, 61–63.

23 Presley, J. T., Carns, H. R., Taylor, E. E. and Schnathorst, W. C. 1966. *Phytopathology*, **56**, 375.

24 Redemann, C. T. and Meikle, R. W. 1958. *Adv. Pest Control Res.* **2**, 183–206.

25 Schnathorst, W. C. and Mathre, D. E. 1966. *Phytopathology*, **56**, 1155–1161.

26 Staron, T. and Allard, C. 1964. *Phytiatrie-Phytopharmacie*, **13**, 163–168.

27 Staron, T., Allard, C., Darpoux, H., Grabowski, H. and Kollman, A. 1966. *Phytiatrie-Phytopharmacie*, **15**, 129–134.

28 Summers, L. A. 1968. *Pure and Appl. Chemistry*, **18**, 1–16.

29 Sun, Yun-Pei. 1957. *Adv. Pest Control Res.* **1**, 449–496.

30 Von Schmeling, B. and Kulka, M. 1966. *Science N.Y.*, **152**, 659–660.

31 Wain, R. L. 1959. Royal Inst. of Chemistry Lectures, Monographs and Reports No. 3, 21 pp.

32 Wain, R. L. and Carter, G. A. 1967. *In* D. C. Torgeson *ed* '*Fungicides*', Vol. 1, Academic Press, pp. 561–611.

33 Weltzein, H. C. 1958. *Naturwissenschaften*, **45**, 288–289.

A review of the use of insecticides on the main agricultural crops of the world—3*
Section 3: Cotton

A D Hanna (*the late*)

The world total production of cotton lint is about 11 million metric tons annually and the area is about 34 million hectares distributed as follows (1965):

	× 1,000 *metric tons* (*lint*)	*area* × 1,000 *hectares*
Europe	176	424
North & Central America	4,119	6,703
South America	1,042	5,083
Asia	2,197	11,385
Africa	1,050	4,192
Oceania	19	25
USSR	1,908	2,442
China	1,258	4,654
World Total	11,769	34,908

The principal cotton lint producing countries excluding the USSR and China are:

	× 1,000 *metric tons* (*lint*)	*area* × 1,000 *hectares*
USA	3,256	5,510
India	847	7,827
Brazil	662	4,004
United Arab Republic	520	798
Mexico	577	793
Pakistan	417	1,569
Turkey	325	685
Argentina	138	534
Sudan	150	448
Uganda	79	865
Tanzania	67	223
Nigeria	44	380

* Part 1 of this series appeared in the Summer 1968 issue; Part 2 in the Winter 1968 issue.

No other cash crop appears to suffer from so many species of insect pests as cotton, perhaps because the fruits are produced over a long period of time. The economic production of cotton depends on the efficient control of these insects, but in recommending an insecticide, its effect on the whole complex of insects which attack the crop must be carefully considered.

There is a much closer entomological relationship between the cotton pests in Africa and Asia than those in America. Several African cotton pests are found in Asia with the exception of the red bollworm (*Diparopsis*) which has not been recorded in Asia.

(a) United States of America

The United States of America produce about 3·25 million metric tons or about 27 per cent of the whole world production (1965). It is by far the largest cotton producer in the world.

The main pests of cotton in the United States of America are:

1 Cotton Boll Weevil *Anthonomus grandis* Boh.

The most serious enemy of cotton in America. The eggs are laid in the unopened blossom buds. The weevils and grubs feed on the inner parts of the flower and on the receptacle causing the failure of the flower bud to develop.

Control

The following insecticides are applied every 3 to 7 days until the insect is controlled:

a) BHC e.c. or dust – 0·3 to 0·5 lb gamma acre
b) Aldrin 25 per cent w.p. or e.c. – 1 to 2 lb acre
c) Dieldrin 50 per cent dust or e.c. – 0·3 to 1 lb acre
d) Calcium arsenate dust – 7 to 15 lb acre
e) Endrin 20 per cent dust or e.c. – 1 to 2·5 lb acre

34 Mitchelmore, A. P. G. 1955 Ann. Rept. Section of Ent. Dept. Agric. Uganda. 1954, 114-8

35 Nadkarni, N. T. 1951. 'Bollworms of Cotton in Marathwada Division of Hyderabad State'. *Ind. Cotton Gr. Rev.* **5,** 211-24

36 Owen, W. L. 1956, *Tex. Agric. Expt. Sta.* Prog. Rept. No. 1859, 4 pp.

37 Parencia, C. R. Jr., Cowan, C. B. and Davis, J. W. 1954. 'Insecticides for Cotton Leafworm Control in 1952 and 1953'. *J. econ. Ent.* **47,** 541-2

38 Parencia, C. R., Davis, J. W. and Cowan, C. B. 1957. 'Control of early season cotton insects with systemic insecticides employed as seed treatment'. *J. econ. Ent.* **50,** 31-6

39 Pearson, E. O. 1958. '*Insect Pests of Cotton in Tropical Africa*'. Commonwealth Institute of Entomology, London. 355 pp.

40 Rainwater, C. F. 1952. 'Progress in research on cotton insects'. *Yearboo*

41 Rainwater, C ... in the Effectiver ... *J. econ. Ent.* **44,**

42 Reports Res

43 Reynolds, H ... R. B. 1957. 'Se ... cides'. *J. econ. E*

44 Ripper, W. E. and George, L. 1965. 'Cotton Pests of the Sudan'. Blackwells Scientific Publications, Oxford. 345 pp.

45 Schmutterer, H. 1962. 'On the Economics and Control of the Sudan Bollworm *Diparopsis watersi* Roths with modern insecticides'. (In German) *Z. angew. Ent.* **50,** (1), 110–17

46 Smith, G. L., Richmond, C. A. and Noble, L. W. 1954. 'Mixtures of DDT and other insecticides for control of Pink Bollworm and Boll Weevils in Southern Texas'. *J. econ. Ent.* **47,** 177-8

47 'Spider Mites on Cotton – How to Control them'. 1962. *U.S. Dept. Agric.* Leaflet No. 502

48 Tropical Pesticide Research Unit, Porton Down, Salisbury. Repts. 1960-67

49 Tropical Research Institute, Arusha, Tanzania Repts. 1960-67

... 1962. *U.S. Dept.*

... of DDT on Pink

... oll Weevils in the ... **46,** 1051-3

... rol of Beet Army ... 2-3

KIRBY, A.H.M. 1969

Fungicides for deciduous top fruit: a survey in 1968.

Wld Rev. Pest Control, 8 (1):45-58. [14 tables. East Malling Res. Stn., Kent.]

Microfilm no.

15605

Fungicides for deciduous top fruit: a survey in 1968

A H M Kirby *East Malling Research Station, Kent, England*

Lists of commercial products officially approved for use on various crops are published at intervals by most countries. These contain a proportion of products that were once thought to be adequate but which have in fact been superseded, and often others that are rarely used. A more realistic appraisal of current usage can be obtained by a study of spray programmes and other documents in which firm recommendations are made as to those active ingredients likely to give the best control of particular diseases at particular stages of the season. Such a study has been made for nearly fifty fruit-growing areas of the world, and the author is indebted to extension and advisory officers who sent him programmes and answered queries.

Some limitation of the range of fruit crops to be considered was necessary. The FAO Production Yearbooks list as top fruit: apples, pears, peaches, plums, cherries, and apricots, in that order by weight. The production of apples is greater than that of all the rest added together. Europe produces two-thirds of the apples and pears, three-quarters of the plums and cherries, and nearly half of the peaches and apricots. Although grapes are deciduous perennial plants, they are not included in top fruit; however, as European production alone is nearly as great as total world production of all the top fruits mentioned above (and nearly half as large a tonnage of grapes is also grown elsewhere), fungicide usage is enormous on this crop, and the study has been extended to cover it. No recommendations for the Rhine-Moselle areas nor for France were available, but the products *approved* in Germany and in France for use on this crop have been taken into consideration.

The number of areas making recommendations for fungicide (or bactericide) application at one or more stages in the year are given for various diseases in the above crops at the top of the relevant tables below. The number of areas recommending a particular chemical for a specified disease is listed under the crop or crops that are subject to that disease. It should be remembered, however, that the number of sprays as well as the timing varies greatly from disease to disease, crop to crop, and area to area. Some areas are satisfied with one fungicide for all applications against one disease on one crop or even on related crops; others recommend more than one fungicide for every occasion. At the other extreme, some areas vary the choice as the season progresses so as to enjoy the main advantages, or avoid some of the disadvantages, of certain fungicides at certain stages of host development. The total number of recommendations for the control of any one disease on any one crop may thus far exceed the number of areas concerned.

The need to apply fungicides and other chemicals, including nutrients, at the same time, can lead to problems of incompatibility in the tank and of phytotoxicity on the tree. This may influence the choice of fungicide for a given spray occasion, e.g. several American States warn that captan, dodine, dichlone, sulphur, lime sulphur, and organomercury compounds cannot be used for apple scab control if oil is needed for control of scale insects, and recommend ferbam, thiram or glyodin instead. Phytotoxicity of a fungicide on its own may be a factor limiting its use, and reference will be made to this under individual fungicides. Owing to their rather highly alkaline reaction, lime sulphur and Bordeaux mixtures (especially those containing as much or more lime as hydrated copper sulphate) are incompatible with many other pesticides. This is a further discouragement to their use, but, in spite of the obvious disadvantages, these historic fungicides are still in widespread use, being absent from the tables below only under rusts, pear leaf spots, and the so-called 'summer diseases' of apple and pear.

The reader should bear in mind that recommendations in areas for which no information was available to the author may differ in various ways from those quoted below, and any statement made in this paper must be regarded as limited in this respect.

Scab diseases (Tables 1 and 2)

The group of diseases that cause fruit infections by a *Fusicladium* stage is small but includes the disease for which the largest number of fungicides are recommended, namely, apple scab, which 'down under' is often called black spot. Although cherry scab is rarely seen, pear scab has to be controlled by spraying in all areas covered by this survey, except British Columbia, and the American States of Georgia and Maryland. Peach scab, also known in Australia as freckle, is widespread, especially in eastern North America, but apricot scab is almost confined to Australasia.

The use of copper, either as Bordeaux mixtures or as 'fixed coppers', is confined to bud burst on apple, but on pear Nova Scotia, two areas in Victoria, and New Hampshire recommend Bordeaux in petal-fall and even later sprays; the use of lime sulphur is also confined mostly to pre-blossom although on apple Poland, and on pear South Australia, New Zealand (Alexandra) and eastern Washington, continue to petal-fall. Sulphur is surprisingly rarely recommended for apple or pear, but is still a mainstay on peach, although regarded as unacceptable on apricot almost everywhere. Clearly, great advantage has been taken of the lower phytotoxicity of most organic fungicides, many areas relying on these entirely. The range recommended for pear is smaller than that for apple, but the rise of dithianon and mancozeb on both is notable. The choice on stone fruits is even more restricted, reliance being heavy upon thiram on apricot.

Powdery mildews (Table 3)

Although plum is susceptible to the same powdery mildew that attacks cherry, plum mildew is not of sufficient importance in any of the thirty-six areas for which information was obtained for a fungicide to be recommended. Only eleven of the thirty-three cherry areas regard fungicide treatment as desirable, and almost all of these rely on sulphur. This type of disease is rare also on pear, recommendations being found only for British Columbia, eastern Washington (State), and Tasmania; all three areas favour DNOPC (see p. 56) but only British Columbia recommends

Table 1
Recommended for control of scab (black spot) on apple[1] (in 48 areas) and pear[2] (in 36 areas)

	Apple	Pear
Lime sulphur	19	6
sulphur	8	4
copper	13	17
organomercury	14	2
captan	44	30
folpet	6	—
captafol	2	—
dichlofluanid	1	—
thiram	17	15
ziram	6	10
ferbam	5	8
zineb	6	3
propineb	1	—
metiram	4	—
maneb	2	—
mancozeb	6	5
mancozeb + zineb	1	—
dodine	39	20
glyodin	11	4
dodine + glyodin	2	—
dichlone	6	1
dithianon	7	4
dinitrothiocyanobenzene	1	2

[1]*Venturia inaequalis* (Cke) Wint.
[2]*Venturia pyrina* Aderh.

Table 2
Recommended for control of scab (freckle) on peach[1] (in 26 areas) and apricot[1] (in 9 areas)

	Peach	Apricot
sulphur	15	1
copper	4	–
captan	16	2
thiram	7	8
ziram	2	–
zineb	2	–
dichlone	1	1
dithianon	2	1

[1]*Megacladosporium carpophilum* (Thüm.) Viennot-Bourgin [*Fusicladium carpophilum* (Thüm.) Oudem.]

Table 3
Recommended for control of powdery mildews on apple[1] (in 46 areas), pear[1] (in 3 areas), cherry[2] (in 11 areas), peach[3] (in 16 areas), apricot[3] (in 6 areas), and grape[4] (in 24 areas)

	Apple	Pear	Cherry	Peach	Apricot	Grape
sulphur	34	1	10	12	2	14
lime sulphur	15	2	–	4	–	1*
copper	–	–	–	–	–	12
DNOPC	41	3	–	6	6	4
binapacryl	21	–	–	1	–	–
quinomethionate	17	–	–	2	–	–
folpet	–	–	1	–	–	5

[1]*Podosphaera leucotricha* (Ell. & Ev.) Salm.
[2]*Podosphaera oxyacanthae* (DC.) de Bary
[3]*Sphaerotheca pannosa* (Wallr.) Lév.
[4]*Uncinula necator* (Schw.) Burr.
*in late dormancy only

Table 4
Recommended for control of rusts on apple[1] (in 17 areas), peach[2] (in 11 areas), plum[2] (in 10 areas) and apricot[2] (in 5 areas)

	Apple	Peach	Plum	Apricot
sulphur	–	3	3	2
lime sulphur	–	3	2	–
copper	–	3	3	–
captan	–	1	1	–
dithianon	–	6	4	2
thiram	8	6	2	3
ziram	–	3	2	–
ferbam	15	1	–	–
zineb	6	7	8	3
maneb	3	2	1	–
mancozeb	–	2	1	–
metiram	–	1	1	–

[1] Cedar-apple rust: *Gymnosporangium juniperi-virginianae* Schw.
Quince rust: *G. clavipes* Cke & Pk
[2]*Tranzschelia pruni-spinosae* (Pers.) Diet.

sulphur. This element remains the most favoured fungicide on peach and grape, and is second only to DNOPC on apple. Binapacryl and quinomethionate (formerly oxythioquinox) are fairly widely recommended on apple, where their acaricidal action is advantageous, but not on the other fruits, except on peach in two areas. The powdery mildew disease on grape clearly differs from that on the other fruits in that copper gives good control; products based on copper are needed for downy mildew on this host and this will discourage the use of other products, although the use of folpet has begun in some eastern areas of the USA and may well spread to other areas.

Rusts (Table 4)

Although the rust attacking pome fruits belongs to a different genus from that attacking the stone fruits, both belong to the Pucciniaceae. It is therefore not surprising that recommendations for control of both should be similar. On apple, dithiocarbamates, especially two derived from dimethyldithiocarbamic acid, are exclusively chosen, and fungicides of these two groups are also favoured on stone fruits. The use of lime sulphur and Bordeaux mixture on peach and plum is limited to Australia; the latter fungicide is only advised at bud burst or white bud, but so-called dry mix lime sulphur is recommended in cover sprays in all parts of Victoria on both hosts. Queensland and South Africa include sulphur on peach; the votes for this fungicide on plum and apricot come from South Africa and Spain, but even in Spain this recommendation appears to be restricted to one variety that will stand it.

The popularity of dithianon for use on stone fruits and its absence from the recommendations for apple and pear seem to be almost entirely related to the world distribution of the diseases and of the chemical. Apple rust is confined to North America where dithianon has not (as far as the records show) been tested, whereas stone fruit rust is restricted to the southern hemisphere, including Australia where dithianon is already very popular, and to certain European areas (plus Israel). The only area where a fungicide is recommended against rust on pear is Bavaria; here zineb is recommended although dithianon is a German product.

In Israel, captan is recommended for Japanese plums only, and zineb for European plums, but metiram may be used there on all plums. Norway has a shot hole disease that is caused by *Pucciniastrum areolatum*; ferbam is recommended for control.

Bitter and black rots (Table 5)

The disease perhaps most widely known as bitter rot is one of the closely allied group that cause rotting of pome fruits, especially apples, in storage, but also lead to perennial

Table 5
Recommended for control of bitter rot (ripe rot, bull's-eye rot, or target spot)[1] on apple (in 35 areas) and pear (in 3 areas) and of frog-eye leaf spot (black rot)[2] on apple (in 14 areas)

	Bitter rot		Black rot
	Apple	Pear	
captan	32	2	10
folpet	9	–	6
dichlofluanid	1	–	–
thiram	10	1	3
ziram	7	1	–
tecoram	1	–	–
ferbam	–	–	6
dithianon	1	2	–
dodine	–	–	2
dichlone	–	–	1

[1]Variously ascribed to: *Glomerella cingulata* (Stonem.) Spauld. & Schrenk; *Pezicula* spp.; *Gloeosporium* spp.; *Neofabraea* spp.
[2]*Physalospora obtusa* (Schw.) Cke

Table 6
Recommended for control of 'summer diseases' on apple[1] (in 16 areas) and pear[2] (in 4 areas)

	Apple	Pear
zineb	15	2
thiram	1	–
ziram	–	1
ferbam	–	1
captan	3	3
folpet	11	–
glyodin	1	1

[1]A group of diseases (see text for list)
[2]Sooty blotch: *Gloeodes pomigena* (Schw.) Colby

cankers on the tree. No mention of control is made in programmes from eastern Canada, Wisconsin and South Africa. Some areas, for example Oregon, distinguish two diseases on apple: anthracnose, due to *Neofabraea malacorticis*, and perennial canker, due to *N. perennans*. In the United Kingdom, two organisms are also implicated: *Gloeosporium perennans* Zeller & Childs, and *G. album* Osterw. So far, spraying has only been of value for the protection of the fruits shortly before harvest. The sulphenamide group of fungicides is by far the most widely favoured, with dimethyldithiocarbamates next. On pear, where this disease is much rarer, dithianon is recommended in northern Europe, though not in the UK.

Black rot, which also causes frog-eye spot on foliage, is confined to apple and to USA east of the Rocky Mountains. Georgia advises lime sulphur, and Indiana Bordeaux mixture, in the dormant season; otherwise the choices are very similar to those for the *Gloeosporium* group of diseases, except that dodine and dichlone receive a little support.

Other diseases on apple and pear (Tables 6 and 7)
The areas that have to spray against black rot also have several other diseases on apple that must be controlled by fungicides. These are grouped together as the so-called 'summer diseases', and are adequately controlled by some of the chemicals in use for scab control, notably folpet. However, most areas prefer to add zineb for control of sooty blotch (due to *Gloeodes pomigena* (Schw.) Colby), especially in the last cover sprays. This disease occurs also on pear; Ontario recommends zineb, captan or glyodin and New Jersey zineb, captan or ferbam, but Pennsylvania prefers thiram. Other apple diseases controlled by the normal summer fungicide programme in eastern USA and Ontario include Botryosphaeria rot (due to *Botryosphaeria ribis* Gros. & Dug.), Brooks fruit spot (due to *Mycosphaerella pomi* (Pass.) Lind.), fly speck (due to *Microthyriella rubi* Petr.), and blotch (due to *Phyllosticta solitaria* Ellis & Everh.).

Two diseases that call for fungicidal treatments occur on pear only. Leaf fleck, due to *Mycosphaerella sentina* (Fr.) Schroet., which has an imperfect stage known as *Septoria piricola* Desm., attacks the foliage early in the season. It is clear from the list of chemicals recommended (Table 7) that control will commonly be achieved by spraying for control of scab. Later in the season, quince leaf blight, due to *Fabraea maculata* Atk., may cause spotting of the foliage, but again normal scab control programmes will give protection. New York State claims clearly that both diseases occur there; other USA areas refer only to Fabraea spot, while Spain refers only to septoriosis (presumably due to *M. sentina*).

Two diseases affecting pear blossom are due to bacteria: blossom blight or blast due to *Pseudomonas syringae* van

Table 7
Recommended for control of leaf spot (fleck or blight)[1] on pear (in 14 areas)

captan	12
captafol	1
dodine	4
sulphur	4
ferbam	7
thiram	3
ziram	2
zineb	3

[1]Leaf spot (fleck, quince leaf blight): *Fabraea maculata* Atk.
Leaf fleck: *Mycosphaerella sentina* (Fr.) Schroet. [*Septoria pyricola* Desm.]

Hall (= *Ps. prunicola* Wormald) and fireblight, *Erwinia amylovora* (Burrill) Winslow *et al.* In the Auckland and Hawke's Bay districts of New Zealand, blast is considered the more serious disease, and Bordeaux mixture[1] at 10:8:100 is recommended at bud swelling and at 6:8:100 at bud burst and three times during leaf-fall; application at 10:8:100 is advised at monthly intervals throughout the winter, but it seems doubtful if this can do any good and could increase the risk of copper poisoning of the soil. Leaf-fall application of copper is also recommended in the Hood River Valley of Oregon and in Bavaria; in northern Italy, a bud burst application against scab is said to be effective against *Ps. syringae.* Fireblight is controlled by bloom sprays of 1:1:100 Bordeaux mixture or a fixed copper in British Columbia, even though it is admitted that copper cannot be applied with safety on the variety Beurré d'Anjou; eastern Washington also relies exclusively on copper but adds a post-harvest treatment. Of the eighteen other areas subject to fireblight, seven advise several sprays of streptomycin at up to 100 ppm during the bloom period; the remainder recommend streptomycin but alternatively a weak Bordeaux mixture except on d'Anjou and Doyenné du Comice. Apart from California, spraying against fireblight on *apple* is confined to fifteen eastern States of the USA. All these areas recommend streptomycin in bloom, but two add copper in the dormant period and four suggest very weak Bordeaux mixture as an alternative in bloom.

[1]All proportions of copper sulphate: hydrated lime are given for 100 Imperial gallons

Twig and stem cankers, due to *Nectria galligena* Bres., occur in some areas on apple and pear. Rigorous cutting out is essential, but a few fungitoxic chemicals are helpful. North Germany advises adding a copper and a mercury preparation to the DNOC–petroleum oil spray in March (late dormancy), whereas Oregon recommends Bordeaux mixture, 9:9:100 in the autumn, and the Netherlands favour two applications during leaf-fall of a mixture of copper and mercury. Wounds left after cutting out on apple trees should be painted with an organomercury-in-oil preparation; this is probably unwise on pear varieties sensitive to organomercury fungicides. The Netherlands prefer a captan paste even on apple. A preparation based on *ortho*-phenylphenol is under trial.

Copper is so far the only fungicide of value for control of collar or crown rot, due to *Phytophthora cactorum* (Lebert & Cohn) Schroet. and/or *P. syringae* (Kleb.) Kleb. Infected areas of bark should be cut out and the wounds protected by a slurry made from dry Bordeaux mixture powder; the soil should be drenched with copper sulphate or oxychloride.

The Milton–Freewater area of Oregon advises spraying apple trees, after pruning, with Bordeaux mixture or fixed copper plus petroleum oil to protect against a disease caused by the wood-rotting saprophyte, *Polystictus versicolor* (L. ex Fr.) Fr. (= *Polyporus versicolor*).

Stone fruit diseases

Apart from powdery mildews and rust, which have been dealt with above, there are several diseases that are either confined to stone fruit species or are more readily (or economically) dealt with by fungicide treatments than they are on pome fruits. All four stone fruit species of wide economic importance (peach, cherry, plum and apricot, in that order) are very subject to two diseases; these are shot hole disease or Coryneum blight, due to *Clasterosporium carpophilum* (Lev.) Aderh. (= *Coryneum beijerinckii* Oud.), and brown rot, due to *Sclerotinia* spp. of which *S. laxa* (= *Monilinia laxa*) causes blossom wilt on stone and pome fruits. Apart from northern Italy, where copper at bud burst is followed right through to harvest by zineb on apple and ziram on pear, and in Norway, where calcium arsenite is recommended for the dormant stage and captan in bloom on pear, no attempt seems to be made anywhere

3rd 1969, with two plenary lectures and on the following two days there will be four sessions each comprising four invited papers followed by a general discussion guided by an expert panel. The invited papers will be preprinted and distributed before the meeting. Session 1 will examine how future changes in the economics of crop protection and pest control will affect the pattern of pesticide usage; invited papers will deal with technological forecasting, the effects of changes in agricultural practice and food technology, the discussion will be on these topics and include the effects of legislation. Session 2 involves the effects of technological developments in conventional pesticides and their application, in biological and integrated control and in the modification of plant growth by applied chemicals; discussion will include these topics and the need for new developments. Session 3 will examine the internal technological and economic problems of the pesticide industry with papers on the derivation of new pesticides, their testing and marketing. The final session has the broad title 'The Challenge of the Future to the Pesticide Industry' serving to round off previous discussions and to examine such problems as those arising from the long development period of new pesticides, the adequacy of present patent legislation and the special case of minor crops.

Further particulars may be obtained from the Hon. Secretary Pesticides Group, Dr M. B. Green, P.O. Box 13, Imperial Chemical Industries Ltd, The Heath, Runcorn, Cheshire.

1969

DEKKER, J.

Acquired resistance to fungicides.

Wld Rev. Pest Control, 8 (2):79-85. [1 fig., 3 diag., 1 graph, 1 table. 19 ref. Phytopath. Lab., Wageningen, Netherlands.]

Microfilm no. 15606

Acquired resistance to fungicides

J Dekker *Laboratory of Phytopathology, Wageningen, Netherlands*

Introduction

The emergence of resistance to antibiotics in bacteria and to pesticides in insects has caused problems in medicine and agriculture. The development of resistance in fungi to fungicides, however, has been much less frequently encountered under conditions of practical use. Most of our common protectant fungicides are relatively non-specific in their mode of action and probably react with numerous biochemical systems. If such a fungicide enters the fungal cell without being detoxified, the development of a high degree of resistance cannot be expected.

The danger of acquired resistance in plant pathogens, however, should not be underrated. A high degree of resistance to fungicides, belonging to the so-called aromatic hydrocarbon group, seems to arise rather easily, and increased resistance of certain plant pathogens to organic mercury- and cadmium compounds in the field has been reported recently. Moreover it should be realized that the development of more selective fungicides in the future might enhance the possibilities for the parasite to acquire resistance.

It is not intended to give a survey of all laboratory research on this subject. After a few remarks on the occurrence of acquired resistance to commercial fungicides the discussion will be limited to mechanisms of resistance, which may be operative in fungi. These are illustrated with a few selected examples, for the larger part derived from research with experimental fungicidal compounds.

Importance in practice

Resistance to fungicides of the aromatic hydrocarbon group, such as diphenyl, sodium orthophenylphenate, quintozene (PCNB), may develop in the laboratory as well as under conditions of practical use (Georgopoulos and Zaracovitis, 1967). Diphenyl resistance became a major problem in the control of *Penicillium* rot of citrus during transport and storage. Resistance to one member of the group usually was accompanied by reduced sensitivity to other members. Therefore, it became necessary to discontinue the use of sodium orthophenylphenate in washing the fruit and to search for other vapor phase fungistats to replace diphenyl. Resistance to PCNB has been reported for various fungi, a.o. *Rhizoctonia solani*. Failure of hexachlorbenzene to control common bunt of wheat was described by Kuiper (1965).

Georgopoulos (1966) demonstrated that resistance to aromatic hydrocarbons in *Hypomyces solani* f. *cucurbitae* resulted from single gene mutations, and that mutation could take place at at least five loci. The mechanism of resistance was studied. Eckert (1962), who investigated 19 derivatives of nitrobenzene, considered these compounds as non-specific toxicants and suggested that impaired permeability might be a cause of resistance. In that case, however, it would be difficult to understand that some resistant fungi require the toxicant for growth, as was shown for PCNB and *Sclerotium rolfsii* (Georgopoulos, 1964). Investigations with the fungus *Hypomyces solani* f. *cucurbitae* showed that diphenyl interfered with at least two transport processes at the protoplast membrane, reducing uptake of phosphate and stimulating potassium transport. It appeared that mutants of this fungus possess a potassium transport system, which is insensitive to the stimulatory influence of diphenyl. Evidence was obtained that genes responsible for resistance control permeability phenomena as well as other properties of the cell (Georgopoulos *et al.*, 1967).

In this connection it is of interest to note that fungal species resistant to aromatic hydrocarbons are tolerant also to polyene macrolides, antibiotics which interfere with cell permeability. These act upon certain sterols present

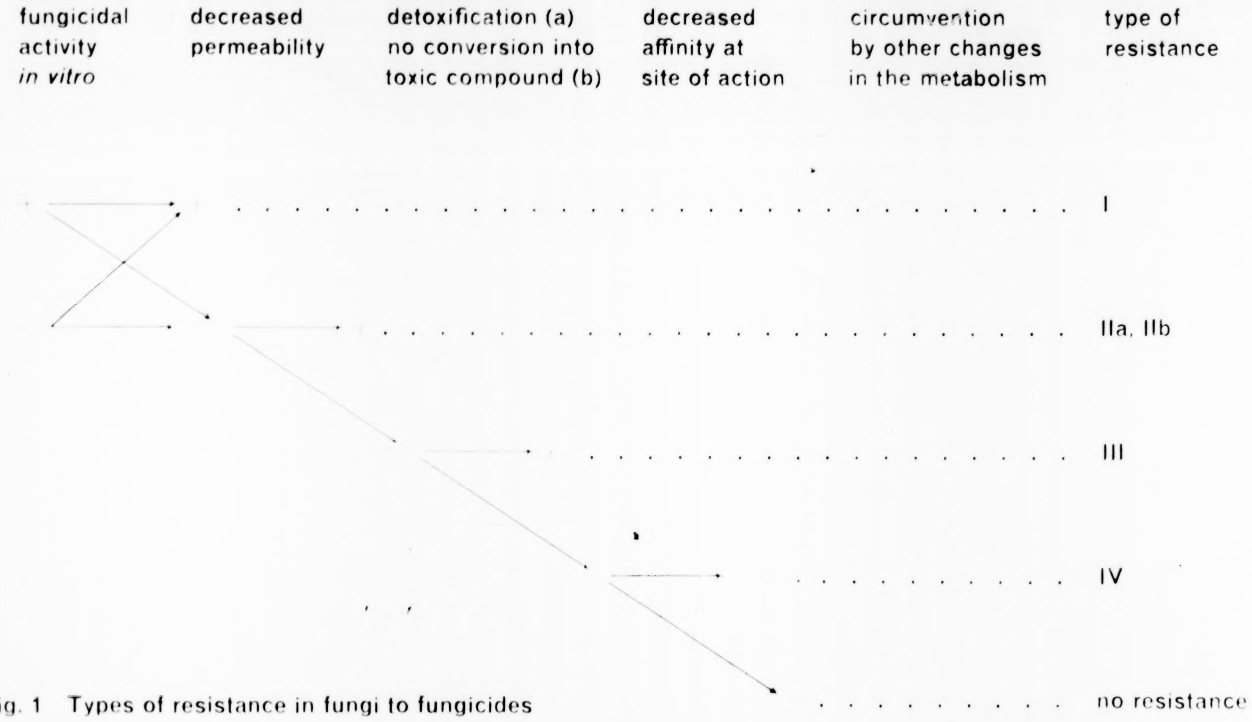


Fig. 1 Types of resistance in fungi to fungicides

in the protoplast membrane, and cause leakage of vital cell constituents. Organisms which lack these sterols in the membrane, such as fungi belonging to *Pythium* and *Phytophthora* species, are resistant to these antibiotics. A bursting of the cell after exposure to the fungicide has been observed not only with some aromatic hydrocarbons, but also with polyene macrolides (review Dekker, 1968a, p. 600). The mechanism of action of the aromatic hydrocarbon fungicides, and the physiological and biochemical background of the resistance in mutants still needs further elucidation.

Development of resistance in the field to metal toxicants has not been noticed until recently. Noble *et al.* (1966) and Malone (1968) found resistance to organic mercury compounds in *Drechslera avenae* (*Pyrenophora avenae*) on Scottish seed oats. These strains, isolated from the field, showed increased tolerance to organic mercury also in the laboratory. Germination of conidia normally is inhibited at 7·5–15 ppm of ethyl mercuric chloride, but the conidia of the Scottish isolates germinated even at 25 ppm. Recently, also, in Holland, strains of this fungus showing increased tolerance to organic mercury compounds were isolated from oat seeds. Resistance was found only to the compounds which contained an aryl radical and not to those with an alkyl radical. The development of resistance in turf grass pathogens to cadmium compounds has been reported in the USA. *Sclerotinia homoeocarpa*, causing dollar spot, appeared unsuccessfully controlled by dosages, which in earlier years provided adequate control (Cole *et al.*, 1968). In none of the above mentioned cases has the mechanism of resistance been elucidated. Decreased permeability or detoxification may play a role.

Mechanisms of resistance

According to the mechanism of resistance, various types of resistant fungi may be distinguished as is shown in fig. 1.

Decreased permeability

The antibiotic blasticidin-S, discovered in 1958 in Japan in the culture broth of *Streptomyces griseochromogenes*, inhibits growth of *Piricularia oryzae* at concentrations of 5 to 10μg per milliliter. The probable site of action of blasticidin-S was considered to be the final step of protein synthesis. Resistant strains of *P. oryzae* were obtained, which tolerated 1000–4000 μg per milliliter of the antibiotic. In cell free extracts from these resistant strains, however, blasticidin-S did inhibit protein synthesis. From this it was concluded that permeability might play a decisive role in the resistant clones (Huang *et al.*, 1964).

Parry and Wood (1959) obtained strains of *Botrytis cinerea* tolerant to captan by repeated transfer of this fungus to media containing higher concentrations of captan. They suggest that the permeability of the fungal cell for captan was decreased. The increased tolerance was very limited and it was lost again after transfer of the fungi to media free of toxicant.

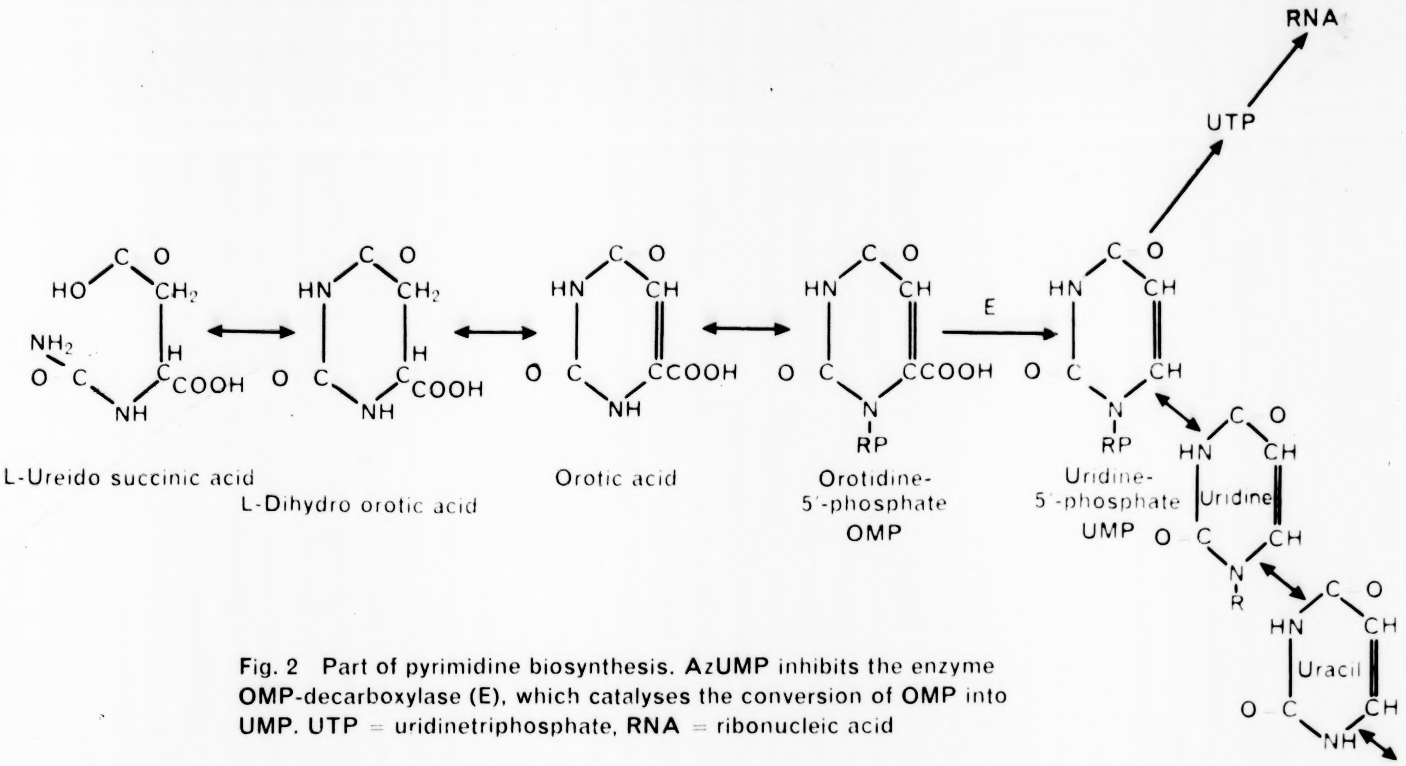


Fig. 2 Part of pyrimidine biosynthesis. AzUMP inhibits the enzyme OMP-decarboxylase (E), which catalyses the conversion of OMP into UMP. UTP = uridinetriphosphate, RNA = ribonucleic acid

Detoxification

Detoxification of a fungicide may occur by a modification of its molecule with a concomitant loss of fungicidal activity after entrance into the cell, or by a binding of the fungicide to other cell constituents than those involved in the inhibition. The mercury tolerance of *Aspergillus niger* has been explained in the latter way by Ashworth and Amin (1964). This fungus possesses an intracellular pool of non-protein sulphhydryl compounds that protects enzyme systems by forming complexes with mercury as soon as it is taken up by the thallus. Fungi with much less protein-SH, such as *Rhizoctonia solani* and *Pythium ultimum*, are extremely sensitive to mercury compounds. The resistance of *Fusarium lycopersici* to ascochitin appears to be due to a reduction of this antibiotic by the fungus to the less fungitoxic dihydroderivative (Oku and Nakanishi, 1964). These examples, however, concern an existing difference in sensitivity between various genera of fungi; it is not known whether the development of resistance in a normally sensitive fungus might be due to such a phenomenon.

Decreased conversion into a fungitoxic compound

As a counterpart of detoxification the phenomenon of conversion of an inactive compound into a fungicide by the fungus (lethal synthesis) might be mentioned.

6-Azauracil (AzU) inhibits spore germination and growth of the fungus *Cladosporium cucumerinum*. This inhibition is not due to a direct action of this compound on the fungus, but to 6-azauridine-5′-phosphate (AzUMP) which is synthesized by the fungus from AzU via 6-azauridine (AzUR) in the following way:

$$\text{AzU} \underset{\text{phosphorylase}}{\overset{\text{uridine}}{\longleftrightarrow}} \text{AzUR} \underset{\text{kinase}}{\overset{\text{uridine}}{\longleftrightarrow}} \text{AzUMP}$$

AzUMP inhibits orotidine-5′-phosphate decarboxylase, an enzyme which functions in the pyrimidine biosynthesis *de novo* (E in fig. 2).

By irradiation with ultraviolet a strain of the fungus was obtained (strain III), which appeared resistant to AzU, but not to AzUR or AzUMP. It appeared from paper chromatographic studies with AzU-2-^{14}C that the AzU resistant strain was unable to convert AzU to AzUR, so that the final toxic product, AzUMP, could not be formed (fig. 3). The resistance was apparently due to a lack of activity of the enzyme uridine phosphorylase. In the same way these strains appeared unable to convert the normal metabolite uracil into uridine (Dekker, 1967).

Decreased affinity for the inhibitor at the reactive site

Strains of *Saccharomyces cerevisiae* which were resistant to the antibiotic cycloheximide were obtained from normally sensitive organisms by gene mutations (Wilkie and Lee, 1965). The action of this antibiotic on the wild strain is due to the inhibition of the transfer of amino acids from

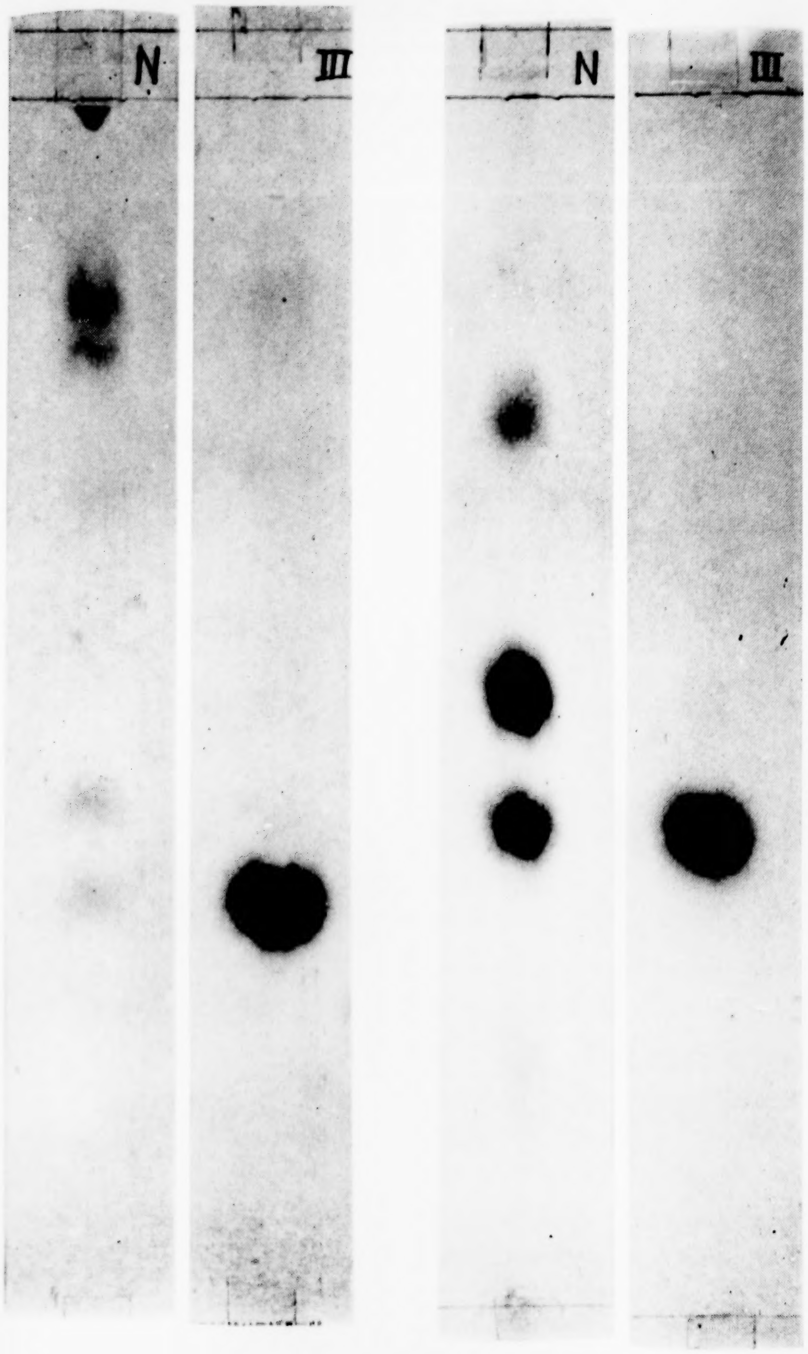


Fig. 3 Conversion of 6-azauracil-C^{14} (left) and uracil-C^{14} (right) by an AzU-resistant strain (III) and a wild strain (N) of Cladosporium cucumerinum. The resistant strain lacks the capacity to convert 6-azauracil and uracil into other compounds (from Dekker, 1968, by permission of Netherlands Journal of Plant Pathology).

amino acyl transfer RNA to the nascent polypeptide chain on the ribosome. Cycloheximide impedes the movement of ribosomes along the messenger RNA, so that no incorporation of amino acids takes place. Some of the cycloheximide resistant mutants appeared freely permeable to the drug; therefore the mechanism of resistance was searched for inside the fungal cell. Cooper *et al.* (1967) studied the incorporation of amino acids into protein in cell free systems of wild and resistant strains, carrying the recessive resistance gene $ac^r{}_8$. *In vitro* the incorporation of amino acids was inhibited by cycloheximide in the wild strain, but not or at least much less in the resistant strain. They then combined ribosomes and supernatant from sensitive and resistant strains. When the ribosome fraction from the wild strain was incubated with the supernatant of the resistant strain, no resistance to cycloheximide was observed; when, however, the ribosome fraction of a resistant strain was combined with the supernatant of a wild strain, resistance to cycloheximide was obtained (fig. 4). It was therefore concluded that it was the ribosomes which conferred resistance to cycloheximide in the cell free system, and not the supernatant fraction.

When the effect of an inhibitor depends on its affinity to one particular enzyme, then a lack of affinity to this inhibitor will be accompanied by resistance. Such a case has been reported by Lewis (1963), working with the fungus *Coprinus lagopus*, which is sensitive to ethionine. He obtained mutants with increased resistance to ethionine. It appeared that ethionine entered the cells of the resistant strains as well as those of sensitive strains, and that the resistance was located inside the cell. Lewis was able to provide a very elegant hypothesis for the ethionine resistance in one of the resistant mutants. First a few words about the mechanism of action of ethionine. It is known to be an antimetabolite of methionine, an amino acid which may be incorporated in protein or may serve as a methyl donor to several other compounds. In order to function in one of these two ways, methionine has to be 'activated' in the presence of ATP and the so-called 'methionine activating enzyme' (fig. 5). This enzyme may also activate ethionine, which leads to incorporation of ethionine into protein, or to the formation of S-adenosyl ethionine, which acts as a donor of ethyl groups instead of methyl groups. The latter seemed to have the primary inhibitory effect. It appeared that the above mentioned mutant strain was unable to activate ethionine, but had retained the capacity to activate methionine. Apparently the methionine activating enzyme in the resistant mutant differed slightly from that in the wild strain, resulting in a lack of affinity to ethionine, but not to methionine. This was explained as the result of a mutation in the structural gene, which forms the messenger RNA coding for this enzyme.

Circumvention by other changes in the metabolism

When a particular enzyme is the primary site of attack by a fungicide, increased tolerance may be the result of the ability of the organism to compensate for the effect of the

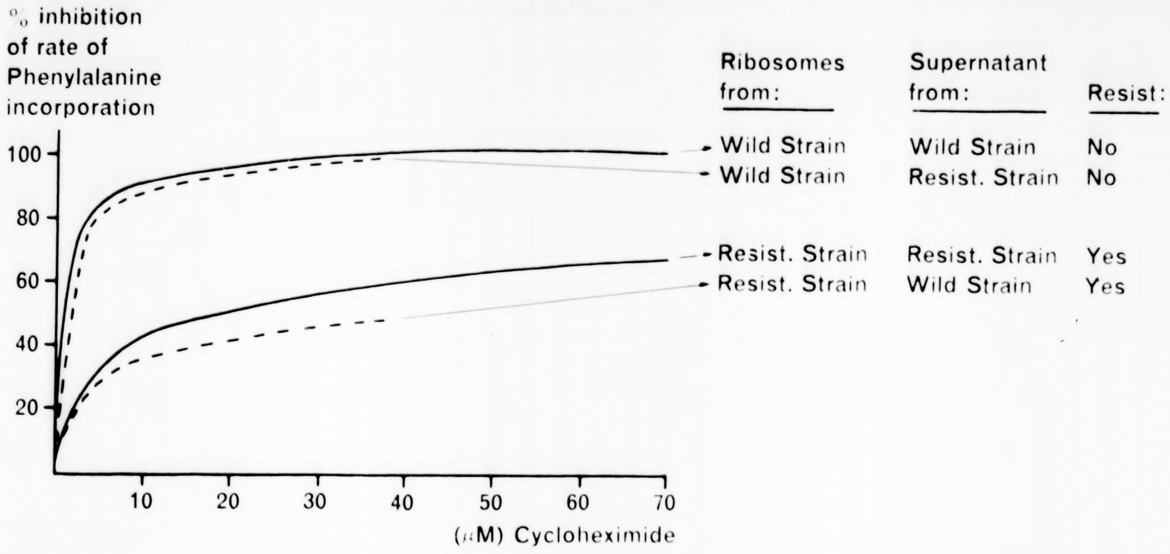


Fig. 4 Inhibition of phenylalanine-C^{14} incorporation by cycloheximide in cell free systems containing ribosomes and supernatant from a cycloheximide resistant and a wild strain of Saccharomyces cerevisiae (redrawn from Cooper *et al.*, 1967, by permission of Journal of Molecular Biology).

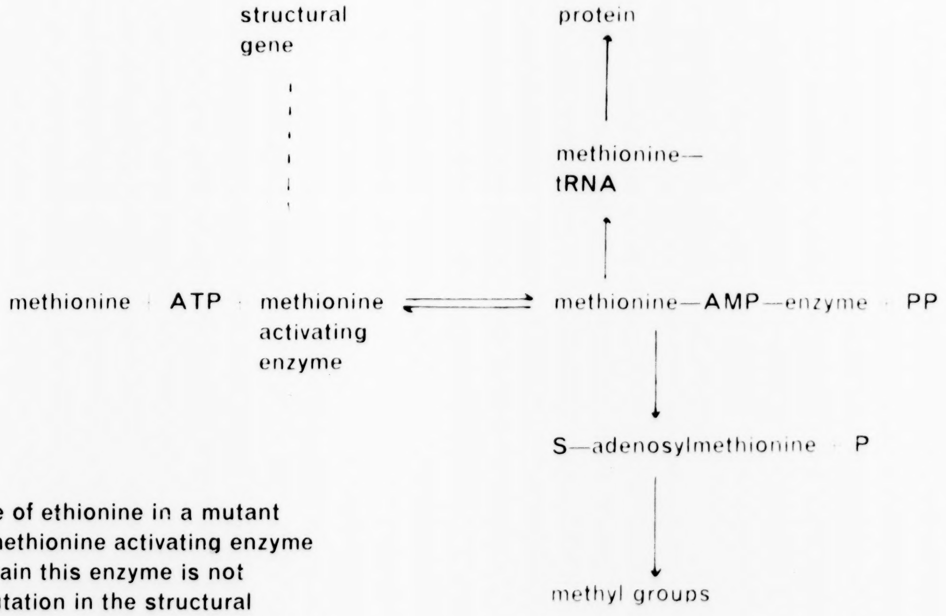


Fig. 5 Hypothesis to explain the resistance of ethionine in a mutant of *Coprinus lagopus*. In the wild strain the methionine activating enzyme also activates ethionine; in the resistant strain this enzyme is not capable of activating ethionine, due to a mutation in the structural gene coding for the enzyme (according to data from Lewis, 1964, by permission of Nature, London).

inhibition, for example, by increasing the production of this enzyme, or to circumvent the inhibited spot by an alternate pathway. Evidence for this was obtained from experiments with an AzU resistant strain of *Cladosporium cucumerinum* (strain R). The antifungal activity of the antimetabolite AzU depends, as has been mentioned above, on its conversion into AzUMP, which inhibits the enzyme orotidine-5′-phosphate (OMP) decarboxylase (fig. 2). This AzU-resistant strain R also appeared resistant against AzUR and AzUMP, so that its mechanism of resistance could not be explained by lack of conversion of AzU to AzUMP, as was the case with strain III of *C. cucumerinum* mentioned earlier in this article. Further, AzUMP did inhibit decarboxylation of OMP in strain R to the same degree as in the wild strain, without inhibition of spore germination or mycelium growth (table 1). Normal growth of this resistant strain even occurred at a concentration of AzUMP, which was at least ten times as

Table 1
Effect of 4.10^{-4} M 6-azauridine-5′-phosphate (AzUMP) on spore germination, growth of germ tubes and on activity of orotidine-5′-phosphate (OMP)-decarboxylase in strains of *Cladosporium cucumerinum*; R: resistant, N: wild; OMP-decarboxylase activity determined by measuring $C^{14}O_2$ liberation from OMP-carboxyl-C^{14} in a conidial suspension; data in % of control without AzUMP. In the resistant strain germination of spores and germ tube growth are not or little inhibited by AzUMP in spite of complete inhibition of OMP-decarboxylase

	strain R	strain N
germination of spores	100	28
length of germ tubes	88	7
OMP-decarboxylase activity	< 1	< 1

high as the concentration which completely inhibited the decarboxylation of OMP (Dekker, 1968b).

Preliminary experiments indicate that the resistant fungus may circumvent the inhibited spot by an alternate pathway. The nature of this pathway needs further elucidation.

Survival of mutants on the plant

Little is known about the survival of fungicide resistant mutants on the plant. Do they only survive by the fact that the fungicide eliminates the fungicide-sensitive strains, or do they possess competitive ability even in the absence of the fungicide? A preliminary experiment has been carried out with an AzU-sensitive strain of *C. cucumerinum* and the above mentioned resistant strain. Cucumber seedlings were inoculated in the greenhouse with a 50:50 mixture of conidia obtained from the resistant and the wild strain. The plants were not treated with AzU. After a week, when sporulation was abundant, the conidia were harvested from the seedlings, and the sensitivity to AzU determined in a spore germination test. It appeared that the ratio between resistant and sensitive conidia had shifted in favour of the resistant strain. This trend continued in the four following weeks of the experiment, when the conidia harvested were again used for inoculation (to be published). This emphasizes the difficulties which may be encountered when resistance to selective fungicides does appear in the crop.

Concluding remarks

Our knowledge of the mechanism of action of most fungicides is, in spite of excellent research in various parts of the world, still scanty. As a consequence, the mechanism of resistance to agricultural fungicides, as found in the laboratory and in the field, is still obscure in most cases. Therefore a number of experimental fungicides, not in practical use against plant diseases, have been included in this review.

It is not likely that a high degree of resistance will develop in practice against agricultural fungicides, which act as multisite inhibitors. Some increase in tolerance may occur due to impaired uptake by the fungal cell or to detoxification. This may lead to practical problems, when the margin between fungitoxic and phytotoxic concentrations is small, so that it will be impossible to raise the dose significantly. A much higher level of resistance, however, has been found in laboratory experiments with selective fungicides, which act at a specific site in the metabolism of the cell. This should be taken into account when the use of agricultural fungicides shifts from predominantly multisite inhibitors to more selective fungicides. The lack of data, however, of the development and survival of highly resistant mutants under field conditions, does not yet justify a prediction for the future.

References

Ashworth, L. J. and Amin, J. V., 1964. A mechanism for mercury tolerance in fungi. *Phytopathology* **54**, 1459–1463.

Cole, H., Taylor, B. and Duich, J., 1968. Evidence of differing tolerances to fungicides among isolates of Sclerotinia homoeocarpa. *Phytopathology* **58**, 683–686.

Cooper, D., Banthorpe, D. V. and Wilkie, D., 1967. Modified ribosomes conferring resistance to cycloheximide in mutants of Saccharomyces cerevisiae. *J. Molec. Biol.* **26**, 347–350.

Dekker, J., 1967. Conversion of 6-azauracil in sensitive and resistant strains of Cladosporium cucumerinum. In *Mechanism of action of fungicides and antibiotics*, ed. W. Girbardt, Akademie-Verlag Berlin, 333–339.

Dekker, J., 1968a. Antibiotics. In *Fungicides*, vol. II, 580–635, ed. D. C. Torgeson, Academic Press, New York. In the press.

Dekker, J., 1968b. The development of resistance in Cladosporium cucumerinum against 6-azauracil, a chemotherapeutant of cucumber scab, and its relation to biosynthesis of RNA-precursors. *Neth. J. Pl. Path.* **74**, 127–136.

Eckert, J. W., 1962. Fungistatic and phytotoxic properties of some derivatives of nitrobenzene. *Phytopathology* **52**, 642–649.

Georgopoulos, S. G., 1964. Chlorinated-nitrobenzene-tolerance in Sclerotium rolfsii. *Ann. Inst. Phytopath. Benaki*, N.S. **6**, 156–159.

Georgopoulos, S. G., 1966. Resistance to certain fungicides in Fusarium. *Meded. Landbhog. Gent* **31**, 3: 889–893.

Georgopoulos, S. G. and Zaracovitis, C., 1967. Tolerance of fungi to organic fungicides. *Ann. Rev. Phytopathology* **5**, 109–126.

Georgopoulos, S. G., Zafiratos, C. and Georgiadis, E., 1967. Membrane functions and tolerance to aromatic hydrocarbon fungitoxicants in conidia of Fusarium solani. *Physiologia Plantarum* **20**, 373–381.

Huang, K. T., Misato, T. and Asuyama, H., 1964. Selective toxicity of blasticidin S to Piricularia oryzae and Pellicularia sasakii. *J. Antibiotics* (*Tokyo*) A **17**, 71–74.

Kuiper, J., 1965. Failure of hexachlorobenzene to control bunt of wheat. *Nature, Lond.* **206**, 1219–1220.

Lewis, D., 1963. Structural gene for the methionine activating enzyme and its mutation as a cause of resistance to ethionine. *Nature, Lond.* **200**, 151–154.

Malone, J. P., 1968. Mercury-resistant Pyrenophora avenae in Northern Ireland seed oats. *Plant Path.* **17**, 41–45.

Noble, M., Maggarvie, Q. D., Hams, A. F. and Leafe, E. L., 1966. Resistance to mercury of Pyrenophora avenae in Scottish seed oats. *Plant Path.* **15**, 23–28.

Oku, H. and Nakanishi, T., 1964. Reductive detoxification of an antibiotic, ascochitin, by an insensitive fungus, Fusarium lycopersici. *Naturwiss.* **51**, 538.

Parry, K. E. and Wood, R. K. S., 1959. The adaptation of fungi to fungicides: adaptation to captan. *Ann. appl. Biol.* **47**, 1–9.

Wilkie, D. and Lee, B. K., 1965. Genetic analysis of actidione resistance in Saccharomyces cerevisiae. *Gen. Res.* **6**, 130–138.

Liu, C. F., Liu, W. T. and Chang, C. S., 1966. 'Preliminary report on the development of resistance to dipterex and pyrethrins in *Culex pipiens pallens* Coq. from Shanghai'. *Acta entomol. Sinica*, **15**, 70–2.

Matsumura, F. and Brown, A. W. A., 1961a. 'Biochemistry of malathion resistance in *Culex tarsalis*.' *J. econ. Ent.*, **54**, 1176–85.

Matsumura, F. and Brown, A. W. A., 1961b. 'Biochemical study of a malathion-tolerant strain of *Aëdes aegypti*.' *Mosquito News*, **21**, 192–4.

Matsumura, F. and Brown, A. W. A., 1963a. 'Studies on carboxyesterase in malathion-resistant *Culex tarsalis*.' *J. econ. Ent.*, **56**, 381–8.

Matsumura, F. and Brown, A. W. A., 1963b. 'Studies on organophosphorus-tolerance in *Aëdes aegypti*.' *Mosquito News*, **23**, 26–31.

Mouchet, J., 1966. Tests reported in *WHO Inf. Circ. Insecticide Resist., Insect Behaviour*

Mouchet, J., Elliott, R.
J., 1960. 'La résistance
Wied. et les problèmes
Trop., **20**, 447–56.

Murray, W. D., 1968. '
area characterized by re
Mosquito News, **28**, 233.

Pennington, N. E., 1968. 'Resistance of *Culex tritaeniorhynchus* Giles and *Culex quinquefasciatus* Say to malathion on Okinawa with notes on susceptibility to other insecticides.' *Mosquito News*, **28**, 193–8.

Plapp, F. W. Jr., Bigley, W. S., Darrow, D. I. and Hoyer, R. F., 1965. 'Esterase activity in mosquitoes and its possible relationship to organophosphate and carbamate resistance.' *Mosquito News*, **25**, 30–5.

Rathburn, C. B. Jr. and Boike, A. K. Jr., 1967. 'Studies of insecticide resistance in Florida mosquitos.' *Mosquito News*, **27**, 377–82.

Roblets, E. D., 1965. Tests reported in *WHO Inf. Circ. Insecticide Resist., Insect Behavior and Vector Genetics*, **56**, 24.

Tadano, T. and Brown, A. W. A., 1966. 'Development of resistance to various insecticides in *Culex p. fatigans* Wiedemann.' *Bull. Wld. Hlth. Org.*, **35**, 189–201.

nf. Circ.
56, 25.

nsecticide

'Insecti-
oc. Calif.

SOMERS, E. 1969

Fungicide selectivity and specificity.

Wld Rev. Pest Control, 8 (2):95-100 [1 diag., 1 graph, 2 tables, 44 ref. Dept Natn Health & Welfare, Ottawa, Canada.]

Microfilm no. 15607

Fungicide selectivity and specificity*

E Somers *Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada*

Although the principles of selective toxicity are implicit in all discussion of the mode of action of fungicides it is undoubtedly true that they have received but scant attention, a notable exception being the review by Horsfall and Lukens.[1] It is self-evident that a fungicide must be selective to be effective: it must inhibit or kill a lower plant – that is a fungus – on or within a higher plant. Parenthetically the word *within* appreciably lowers the margin of selectivity available which explains why we have so few systemic fungicides. As Albert[2] has codified so neatly selectivity can be achieved either by:

(i) selective interference with vital processes
(ii) selective accumulation.

It is proposed to consider first these two routes with respect to fungicide selectivity between fungus and plant and then to examine selectivity between fungus and fungus.

Selective interference with vital processes

This route exploits the comparative biochemistry of the host and fungal cell to achieve fungitoxicity without damaging the plant tissue. Although there is a common biochemical ground plan to all living organisms, particularly with regard to the energy-yielding reactions of cells, there are obvious biochemical differences that are responsible for species and organ differentiation. The effectiveness of a very few fungicides depends on these biochemical differences and their mode of action can best be discussed under the following headings.

Cell Wall The outer wall of most fungi has chitin, a polymer of *N*-acetylglucosamine, as the basic building material whereas cellulose forms the structural matrix for higher plants. Therefore, a chitinase could be a possible selective fungicide and it has been shown[3] that chitinase will inhibit germination of *Aspergillus niger* conidia at concentrations as low as 0·1 ppm although an active enzyme does not make a practicable field fungicide.

Griseofulvin was originally suggested to act selectively through its action on chitin.[4] This compound is taken up by plant roots, rapidly transported – unchanged – in the transpiration stream of the host plant, and inhibits the development of the hyphal tip of chitin-forming filamentous fungi:[5] therefore it acts as a systemic fungicide. Of forty plant-pathogenic fungi tested against griseofulvin by Napier *et al.*[6] the five that showed no response were either Oomycetes or fungi in which the filamentous form was absent. However, the mode of action of griseofulvin, particularly since the work of El-Nakeeb *et al.*,[7] cannot now be described simply in terms of chitin and non-chitinous fungi although interference with hyphal wall formation is probably the primary action followed by binding to RNA in sensitive fungi.

It is of interest that Evans and White[8] have recently shown that the antifungal antibiotic radicicolin, which has a similar activity spectrum to griseofulvin, can also produce marked morphogenetic effects in sensitive fungal hyphae. Macris and Georgopoulos[9] have reported that pentachloronitrobenzene – a fungicide particularly active against species with chitinous walls – markedly reduced the hexosamine content of *Neurospora crassa* mycelial walls.

Inhibition of enzyme systems In general, fungicides act as non-specific enzyme poisons.[10] They react indiscriminately with amino and sulphydryl groups, and with essential metals such as copper and iron. Therefore, they are unlikely to show selectivity in their action between fungal and plant enzyme systems. However, Tolmsoff[11] found that the soil fungicide 'Dexon' (sodium *p*-dimethylamino-

*Based on an introductory paper given at the First International Congress of Plant Pathology, London, July 1968.

benzenediazosulphonate) inhibited the mitochondrial oxidation of NADH in *Pythium ultimum* but not in sugar beets. This difference is probably due to the ability of the sugar beet mitochondria to decompose 'Dexon' and it provides a clear example of selectivity.

Antimetabolites Two chemotherapeutants which probably act directly on the fungus can be considered under this heading. The sulphonamides have been shown by Crowdy and Rudd Jones[12] to be transported by plants yet to control only obligate parasites such as rust on bean or oats: their toxicity is antagonized by *p*-aminobenzoic acid which is a normal cell metabolite. Dekker and Oort[13] found that 6-azauracil controlled powdery mildew on cucumber following root application and its toxicity was reversed by uracil and by orotic acid (presumably converted to uracil in the plant). Azauracil does not inhibit germination or penetration of the host but prevents the development of mycelium after the first haustorium. Dekker and Oort consider 6-azauracil to be an antimetabolite of uracil and to be converted to riboside – containing metabolites that subsequently interfere with pyrimidine biosynthesis by inhibiting orotidylic decarboxylase. The important point is that both the sulphonamides and 6-azauracil are only effective against obligate parasites and we know that these fungi have unique nutritional requirements and hence are more susceptible than the host plant to antimetabolite activity.

A number of other biochemical differences between host and fungi have been suggested[14] but these have not yet been used to develop new, practical fungicides. For example, photosynthesis occurs in plants but not in fungi: a much greater proportion of cells in the pathogen undergo cell division than do so in the host, which renders the fungus more susceptible to mutagenic agents. To illustrate the latter example, griseofulvin can arrest mitosis at metaphase in animal tissues and at high concentrations disturbs mitosis in plant cells[5] while 6-azauracil may have a similar effect on powdery mildew fungi. Ergosterol is the major sterol present in most classes of fungi and has not been reported to occur in green plants (although it has been recently found in citrus)[15] so that this biochemical difference could be exploited by a selective fungicide – with exceptions that are considered below.

Selective accumulation by fungi

This process most convincingly explains the mode of action of the great majority of our present-day fungicides. Selective accumulation by fungal conidia as compared with the host plant entails efficient transport of the fungicide to the spore, followed by easy permeability into the spore and eventual storage. There is a wealth of evidence[10] to show that dense suspensions of fungal conidia in aqueous solutions of fungicides rapidly remove the fungicide from the ambient solution either by accumulating the fungicide, as with metal ions, or by decomposing the fungicide molecule, as with many organic fungicides. Table 1 gives some examples from our work at Long Ashton. On a weight basis a high concentration of fungicide – up to 2·4 per cent for dodine acetate with *Alternaria tenuis* – is required for a fungicidal effect.

Table 1
Uptake of fungicides at fungicidal ED_{50} (μg/g spore dry weight)

	Alternaria tenuis	*Neurospora crassa*
Captan[16]	—	6,900
Copper[17]	12,000	2,600
Dodine acetate[18]	24,000	10,000

The question that must be answered is how the accumulation by conidia compares with that of leaves or seeds so as to give the selective margin. Unfortunately few workers have attempted to solve this problem and yet it provides an essential basis to our views on fungitoxic action. Table 2 gives comparative values for the loss of captan from ambient solution – hence calculated uptake – for detached apple leaves and *Neurospora crassa* conidia. It is apparent that the conidia are much more effective in accumulating captan than are apple leaves. Corroboration comes from Silber[19] who showed that captan is appreciably phytotoxic at a concentration of 1 ppm when applied to plant cells lacking a protective cutin coating, i.e. roots of bean and tomato in liquid culture.

Table 2
Uptake of captan from 25 μM solution in 30 minutes[16]

	Uptake (μg/g dry weight)
Apple leaf	1·1
Neurospora crassa conidia	7,100

Some similar information is available for other compounds: Yarwood[20] found that bean rust uredospores absorbed five times as much sulphur as did bean leaves,

Heden and Ulfvarson[21] showed that smut *Ustilago* spores absorbed more methyl mercury dicyanamide than did seeds. All this serves to confirm that plants are protected against foreign molecules by virtue of seed, bark, or cuticle coating. Copper fungicides provide a clear example of this protection mechanism. The phytotoxicity of solutions of copper salts has been known for over a century, yet by formulating copper as compounds of low water solubility plant damage is largely avoided without loss of fungitoxicity. When Burgundy mixture, labelled with radioactive copper, was applied to bean leaves we found that only negligible amounts of copper were sorbed by the leaf surface.[22]

An important aspect of the relative uptake process between leaves and conidia is the rate of fungicide uptake by the fungal cells. This rate varies with the type of fungicide as illustrated in fig. 1 from the plots of typical uptake curves for *N. crassa*, obtained in our Long Ashton work. The cationic dodine acetate shows the rapid rate of sorption and Langmuir type absorption isotherm to be expected with an ionic bonding mechanism. The binding of cupric ions although also an ion-exchange process was slower and did not reach an asymptote: this is because the denatured protein of dead cells is a much better complexing agent for copper than is native protein, so that uptake continues after death. Captan accumulated slowly requiring 50 minutes to reach the fungicidal ED_{50} dose and we have suggested[23] that there is a rate-determining permeability step for, once within the spore, captan reacts rapidly with intracellular thiols.

The speed of fungicide accumulation by the spore is probably a more important overall determinant of fungitoxicity than is specificity of action. For example, cycloheximide is the most potent fungicide on a cell weight basis yet recorded – the ED_{50} against *Saccharomyces pastorianus* being 0·4 μg g cells[24] – and it is a specific inhibitor of protein synthesis, yet the ambient concentration required for fungitoxicity is not very different from that of the completely unspecific copper fungicides.

Sometimes the selective margin for a fungicide between plant and fungus only holds for some genera or even species: copper fungicides are phytotoxic to apple but not to potato foliage; Cox's Orange Pippin and Lane's Prince Albert are sensitive to lime-sulphur while Worcester Pearmain and Bramley's Seedling are unaffected; Evans and Pfeiffer[25] found that 'Dyrene' (2,4-dichloro-6-(*o*-chloroaniline)-1,3,5-triazine) applied to control *Botrytis cinerea* in strawberry was an effective herbicide for creep-

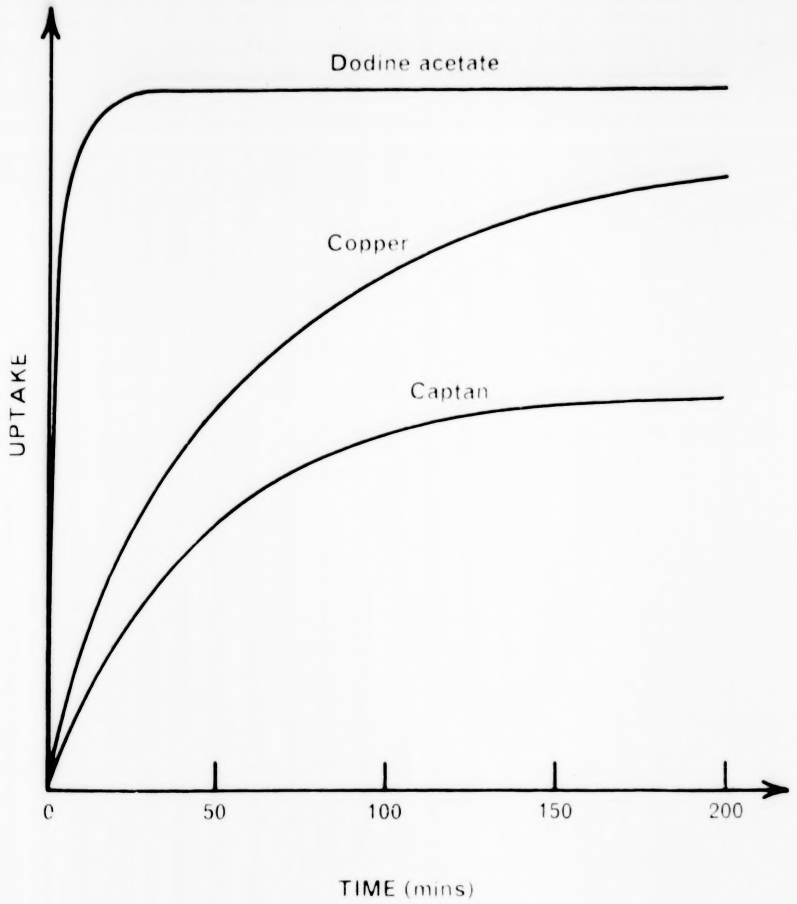


Fig. 1 Typical plots for the uptake of three fungicides by conidia of *Neurospora crassa*

ing thistle; Miller and Gould[26] showed that concentrations of phenyl mercuric acetate that were harmless to *Narcissus* bulbs markedly reduced yields from *Tulipa* varieties.

Many workers have shown that for fungicide molecules with a paraffin tail a selective margin between fungitoxicity and phytotoxicity can be achieved by altering the lipophilic-hydrophilic balance of the molecule. Wellman and McCallan[27] found that for the 2-alkyl-imidazolines maximum fungitoxicity was attained with straight chain substituents containing 17 carbon atoms in the 2-position whereas maximum phytotoxicity occurred with the 11–13 carbon atom derivatives. Van der Kerk[28] reported that tributyltin compounds are about ten times as fungitoxic as triethyltin compounds but the reverse holds for phytotoxicity. Ross and Ludwig[29] showed that for a homologous series of *N*-*n*-alkylethylene thioureas fungistatic activity was greatest with the octyl homologue and phytotoxicity with the amyl. All these examples substantiate the Horsfall hypothesis[30] that an effective fungicide has an active group and a lipid-soluble permeable group, but it must be emphasized that to date this schismatic hypothesis only seems to hold where the lipid group can be identified as a homologous paraffin chain.

Selectivity between fungus and fungus

Under this heading we can discuss those fungicides that have a narrow spectrum of activity, being specific poisons for specific fungi. All that is known of the mode of action of fungicides suggests that as they are usually general poisons specificity will not be a common phenomenon. There are, however, differences and they can be considered in relation to accumulation, detoxication, and membrane permeability.

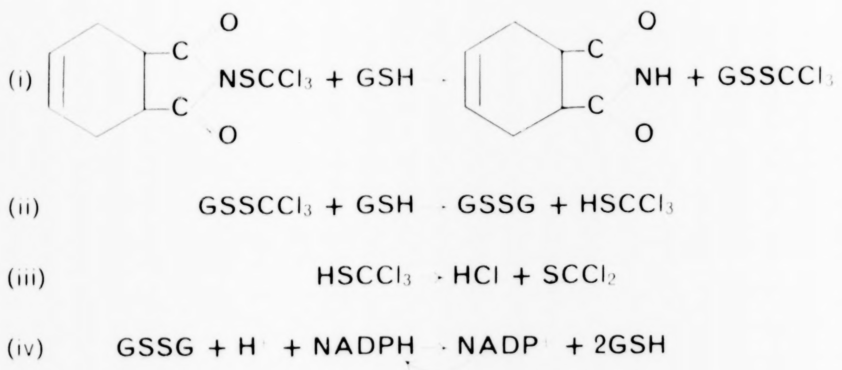
Accumulation If fungicides act by selective accumulation then it would be expected that sensitive fungal species take up more fungicide than do resistant, i.e. resistance based on exclusion. Oomycetes are sensitive to streptomycin and Vörös[31] found that sensitive *Phytophthora* and *Pythium* take up nine times as much streptomycin as do resistant *Mucorales* and tolerant *Aphanomyces* strains. Apparently the insensitive fungi do not detoxify streptomycin. Similarly, El-Nakeeb and Lampen[32] found a correlation between sensitivity to griseofulvin and its uptake by yeasts and mycelia of a number of fungal species.

Low uptake of a fungicide by resistant fungi can be due to membrane impermeability but the absence of internal binding sites is also a possibility. Wescott and Sisler[24] consider that the latter mechanism operates in *Saccharomyces fragilis* – a yeast resistant to cycloheximide – as compared with the susceptible *S. pastorianus*. The different sensitivity of the two organisms to cycloheximide can be explained in terms of the differences in sensitivity of the protein synthesizing systems for it is known that *S. cerevisiae* possesses a ribosomal mechanism of resistance to cycloheximide.[33]

Detoxication If resistant fungi are able to accumulate the fungicide then detoxication could be a mechanism of selectivity. For example, 'Dexon' is ten times as toxic to *Pythium* as it is against *Rhizoctonia*[34] and Tolmsoff[11] found that the mitochondrial system of *Rhizoctonia* decomposed 'Dexon' in the presence of NADH but that this system was absent in *Pythium*. Detoxication may occur in addition to selective accumulation. The antibiotic ascochitine is rapidly sorbed by sensitive fungi (*Cochliobolus miyabeanus* and *Piricularia oryzae*) but not by the tolerant *Fusarium lycopersici*.[35] *F. lycopersici* was found to reduce ascochitine to the less toxic dihydroascochitine. However, when the detoxication mechanism was blocked with sodium azide, *F. lycopersici* became sensitive to the antibiotic. Hence with ascochitine specificity is determined by detoxication not uptake.

Reaction with cellular sulphydryl groups is often invoked as a detoxication mechanism: *Aspergillus niger* with a high thiol content is protected from mercury poisoning as compared with *Rhizoctonia solani* and *Pythium ultimum*.[36] Our work[23,37] with captan has shown that most of the fungicide accumulated by *Neurospora crassa* conidia is detoxified by reaction with the soluble thiol pool, which is largely composed of glutathione. The proposed detoxication scheme is shown in fig. 2. The oxidized glutathione formed from the decomposition of captan (step ii) can be reduced by NADPH in a reaction catalysed by glutathione reductase (step iv): if enzymes capable of reducing $NADP^+$ are present a cyclic regeneration of glutathione can result until all the captan is destroyed. In a comparative study of the total sulphydryl levels of a range of fungi we found[38] a fair relation between sulphydryl content and captan uptake – this latter being a measure of detoxication.

Reaction of Captan with Glutathione



Membrane permeability The clearest examples of fungicides that are known to act primarily on the cytoplasmic membrane are provided by the polyene antibiotics such as nystatin and filipin.[39] These compounds are believed to bind to the cell membrane where they damage the transport system for small molecules such as potassium, sugars, and phosphate. The binding site is considered to be a sterol, possibly ergosterol,[40] that imparts mechanical strength to the membrane perhaps by aligning the phospholipid and protein components. All fungi except the Oomycetes are sensitive to the polyenes and it is significant that sterols have not been found in the mycelia of *Pythium* and *Phytophthora* species. In fact, there is a sterol requirement for the production of oospores by many species of *Phytophthora* and *Pythium*.[41]

The powdery mildew fungicides are classic examples of fungicide specificity in that compounds such as sulphur and dinocap which are effective against *Erysiphe* species give little control of many other fungi. Horsfall[30] has suggested

that mildew fungicides are sufficiently fat-soluble to disrupt the lipid cytoplasmic membrane and hence promote water loss and subsequent death. We examined[42] this hypothesis for a series of captan derivatives all of which contained the trichloromethylthio group, generally regarded as responsible for the toxicity of captan. Although both captan and folpet are equally toxic to *Alternaria tenuis* and *Neurospora crassa*, captan is ineffective against the powdery mildews whilst folpet controls rose mildew (*Sphaerotheca pannosa*). The oleyl alcohol-water partition coefficient of folpet is ten times greater than that of captan which provides a neat confirmation of Horsfall's hypothesis. Unfortunately when a series of six analogues of captan were tested against *Erysiphe graminis* the correlation disappeared. We could find no evidence that lipid solubility was related to the toxicity of the analogues either as eradicants or protectants. *N*-(trichloromethylthio) hexahydrophthalimide was the most effective of the analogues tested and we have evidence (unpublished) that this compound is active in the vapour phase to conidia of *E. cichoracearum* although this has not been confirmed by Zaracovitis[43] in tests against *Oidium begoniae* and *Uncinula necator*. Bent[44] has recently emphasized the importance of vapour phase activity for the control of powdery mildews. The problem is unresolved at present but it is suggested that differences in the rate of uptake of powdery mildew fungicides in the vapour phase will provide the clue to their selectivity.

References

1 Horsfall, J. G. and Lukens, R. J., *Conn. Agric. Exp. Sta. Bull.*, 1966, No. 676.

2 Albert, A., *Selective Toxicity*, 1965, 3rd ed., Methuen, London.

3 Hilborn, M. T. and Farr, W. K., *Phytopathology*, 1959, **49**, 541.

4 Brian, P. W., *Ann. Bot.*, 1949, **13**, 59.

5 Bent, K. J. and Moore, R. H., 'Biochemical Studies of Antimicrobial Drugs', *16th Symp. Soc. gen. Microbiol.*, 1966, 82.

6 Napier, E. J., Turner, D. I. and Rhodes, A., *Ann. Bot.*, 1956, **20**, 461.

7 El-Nakeeb, M. A., McLellan, W. L. and Lampen, J. O., *J. Bact.*, 1965, **89**, 557.

8 Evans, G. and White, N. H., *J. exp. Bot.*, 1967, **18**, 465.

9 Macris, B. and Georgopoulos, S. G., *Phytopathology*, 1968, in press.

10 Somers, E., *Sci. Progr., Lond.*, 1962, **50**, 218.

11 Tolmsoff, W. J., *Phytopathology*, 1962, **52**, 755.

12 Rudd Jones, D., *Outlook on Agric.*, 1956, **1**, 111.

13 Dekker, J. and Oort, A. J. P., *Phytopathology*, 1964, **54**, 815.

14 Cowling, E. B., 'Perspectives of Biochemical Plant Pathology', *Conn. Agric. Exp. Sta. Bull.*, 1963, No. 663, p. 72.

15 Yokoyama, H. and White, M. J., *Phytochemistry*, 1968, **7**, 493.

16 Richmond, D. V. and Somers, E., *Ann. appl. Biol.*, 1962, **50**, 33.

17 Somers, E., *Ann. appl. Biol.*, 1963, **51**, 425.

18 Somers, E. and Pring, R. J., *Ann. appl. Biol.*, 1966, **58**, 457.

19 Silber, G., Ph.D. Thesis, 1957, Cornell Univ., Ithaca, N.Y., 97 pp.

20 Yarwood, C. E., *Therapy of Fungus Diseases*, 1955, Ed. T. H. Sternberg and V. D. Newcomer, p. 130; Little, Brown and Co., Boston.

21 Heden, A. and Ulfvarson, U., *Phytopath. Z.*, 1963, **48**, 397.

22 Somers, E. and Richmond, D. V., *Nature, Lond.*, 1957, **180**, 798.

23 Richmond, D. V. and Somers, E., *Ann. appl. Biol.*, 1966, **57**, 231.

24 Wescott, E. W. and Sisler, H. D., *Phytopathology*, 1964, **54**, 1261.

25 Evans, E. and Pfeiffer, R K., *Nature, Lond.*, 1967, **215**, 782.

26 Miller, V. L. and Gould, C. J., *Phytopathology*, 1967, **57**, 777 and 1202.

27 Wellman, R. H. and McCallan, S. E. A., *Contr. Boyce Thompson Inst.*, 1946, **14**, 151.

28 Van der Kerk, G. J. M., 'Fungicides in Agriculture and Horticulture', *Soc. Chem. Ind. Monograph*, 1961, No. 15, p. 67.

29 Ross, R. G. and Ludwig, R. A., *Can. J. Bot.*, 1957, **35**, 65.

30 Horsfall, J. G., *Principles of Fungicidal Action*, 1956, Chronica Botanica, Waltham, Mass.

31 Vörös, J., *Phytopath. Z.*, 1965, **54**, 249.

32 El-Nakeeb, M. A. and Lampen, J. O., *J. gen. Microbiol.*, 1965, **39**, 285.

33 Cooper, D., Banthorpe, D. V. and Wilkie, D., *J. molec. Biol.*, 1967, **26**, 347.

34 Hills, F. J. and Leach, L. D., *Phytopathology*, 1962, **52**, 51.

35 Oku, H. and Nakanishi, T., *Phytopath. Z.*, 1966, **55**, 1.

36 Ashworth, L. J. and Amin, J. V., *Phytopathology*, 1964, **54**, 1459.

37 Richmond, D. V. and Somers, E. *Ann. appl. Biol.*, 1968, **62**, 35.

38 Richmond, D. V. and Somers, E., *Ann. appl. Biol.*, 1963, **52**, 327.

39 Lampen, J. O., 'Biochemical Studies of Antimicrobial Drugs', 16*th Symp. Soc. gen. Microbiol.*, 1966, 111.

40 Zygmunt, W. A. and Tavormina, P. A., *Appl. Microbiol.*, 1966, **14**, 865.

41 Lilly, V. G., *The Fungus Spore*, 1966, Ed. M. F. Madelin, p. 259, Butterworths, London.

42 Richmond, D. V., Somers, E. and Zaracovitis, C., *Nature, Lond.*, 1964, **204**, 1329.

43 Zaracovitis, C., *Annls Inst. phytopath. Benaki* N.S., 1966, **7**, 193.

44 Bent, K. J., *Ann. appl. Biol.*, 1967, **60**, 251.

Objectives in fungicide testing*

A Tempel, *NV Philips-Duphar, Agrobiological Laboratory 'Boekesteyn' 's-Graveland, The Netherlands*

Speaking about objectives in fungicide testing it is customary to present figures on subjects such as: annual losses of important crops caused by pathogenic fungi, the economics of applying fungicides to prevent those losses, the fungicides usage, and the costs of fungicide development.

I feel myself discharged from that obligation because of the recent publication of a book, 'Fungicides' edited by Dewayne C. Torgeson,[1] which gives all available information on these subjects in the form of excellent contributions by outstanding plant pathologists. I should like to restrict my introductory remarks to a little philosophy about the way we are trying to find new fungicides, without presenting you with hard figures.

Up to now, in plant pathology, fungicides have provided the major and most economic means of crop protection. Apart from copper and sulphur products of long standing, some hundreds[2] of organic chemicals are available to meet the challenge of protecting expanded crop production from losses due to diseases. Generally the application of present-day fungicides under practical conditions provides no problems regarding mammalian toxicity and resistance of the organisms to be controlled.

Moreover these fungicides can be obtained at reasonable prices and I agree with Dr Wellman's statement[3] that one of the most frustrating problems in developing modern commercial fungicides is that it is difficult to find a compound that performs better than those materials already on the market, at a price that makes it economical for the farmer to use. So one could ask: Why go on testing fungicides? And indeed the answer of some people, among them research leaders of industrial firms working in the field of pesticides, is: fungicide research has done its job, it is not worth doing any more. On the other hand many others are continuing extensive research in the field of fungicides, so let us take a closer look at today's source of new fungicides. As a rule, new fungicides, like other pesticides and pharmaceuticals quite generally, are discovered and developed in industrial laboratories, whereas more fundamental research on modes of action is usually conducted in governmental research centres.

For discovering new fungicides the empirical approach must still be used. The techniques involved in the evaluation of chemicals as fungicides have recently been reviewed by Torgeson.[1] There is a trend towards the elimination of *in vitro* methods and the use of practical fungitoxicity tests. As there is a possibility of specificity of fungicidal activity, it is necessary to come as close to the object as is permitted by suitability as a laboratory test; therefore a survey of frequently-used methods can give us an idea of the objectives of fungicide testing. The methods applied in fungicide screens in industry are not regarded as being secret, yet not many surveys of total screens have been published. Without any pretence of exhausting the subject I should like to show some tables representing a number of frequently-used methods.

Foliar protectants

The prime object of the testing of chemicals against powdery mildew is apple powdery mildew, because fruit fungicides are interesting from a commercial point of view; (Table 1); in the second place powdery mildew of cucurbits, cereals, vine and ornamentals is important. Almost every screen comprises at least one test against a powdery mildew, not necessarily because the market for mildewicidal compounds is more interesting than any other separate

*Introductory paper presented at the First International Congress of Plant Pathology, London 14–28 July 1968.

32 El-Nakeeb, M. A. and Lampen, J. O., *J. gen. Microbiol.*, 1965, **39**, 285.

33 Cooper, D., Banthorpe, D. V. and Wilkie, D., *J. molec. Biol.*, 1967, **26**, 347.

34 Hills, F. J. and Leach, L. D., *Phytopathology*, 1962, **52**, 51.

35 Oku, H. and Nakanishi, T., *Phytopath. Z.*, 1966, **55**, 1.

36 Ashworth, L. J. and Amin, J. V., *Phytopathology*, 1964, **54**, 1459.

37 Richmond, D. V. and Somers, E. *Ann. appl. Biol.*, 1968, **62**, 35.

38 Richmond, D. V. and Somers, E., *Ann. appl. Biol.*, 1963, **52**, 327.

39 Lampen, J. O., 'Biochemical Studies of Antimicrobial Drugs', *16th Symp. Soc. gen. Microbiol.*, 1966, 111.

40 Zygmunt, W. A. and Tavormina, P. A., *Appl. Microbiol.*, 1966, **14**, 865.

41 Lilly, V. G., *The Fungus Spore*, 1966, Ed. M. F. Madelin, p. 259, Butterworths, London.

42 Richmond, D. V., Somers, E. and Zaracovitis, C., *Nature, Lond.*, 1964, **204**, 1329.

43 Zaracovitis, C., *Annls Inst. phytopath. Benaki* N.S., 1966, **7**, 193.

44 Bent, K. J., *Ann. appl. Biol.*, 1967, **60**, 251.

1969

TEMPEL, A.

Objectives in fungicide testing.

Wld Rev. Pest Control, 8 (2):101-107. [2 fig., 5 tables. NV Philips-Duphar, 's-Graveland, Netherlands.]

Microfilm no.

15608

Objectives in fungicide testing*

A Tempel, *NV Philips-Duphar, Agrobiological Laboratory 'Boekesteyn' 's-Graveland, The Netherlands*

Speaking about objectives in fungicide testing it is customary to present figures on subjects such as: annual losses of important crops caused by pathogenic fungi, the economics of applying fungicides to prevent those losses, the fungicides usage, and the costs of fungicide development.

I feel myself discharged from that obligation because of the recent publication of a book, 'Fungicides' edited by Dewayne C. Torgeson,[1] which gives all available information on these subjects in the form of excellent contributions by outstanding plant pathologists. I should like to restrict my introductory remarks to a little philosophy about the way we are trying to find new fungicides, without presenting you with hard figures.

Up to now, in plant pathology, fungicides have provided the major and most economic means of crop protection. Apart from copper and sulphur products of long standing, some hundreds[2] of organic chemicals are available to meet the challenge of protecting expanded crop production from losses due to diseases. Generally the application of present-day fungicides under practical conditions provides no problems regarding mammalian toxicity and resistance of the organisms to be controlled.

Moreover these fungicides can be obtained at reasonable prices and I agree with Dr Wellman's statement[3] that one of the most frustrating problems in developing modern commercial fungicides is that it is difficult to find a compound that performs better than those materials already on the market, at a price that makes it economical for the farmer to use. So one could ask: Why go on testing fungicides? And indeed the answer of some people, among them research leaders of industrial firms working in the field of pesticides, is: fungicide research has done its job, it is not worth doing any more. On the other hand many others are continuing extensive research in the field of fungicides, so let us take a closer look at today's source of new fungicides. As a rule, new fungicides, like other pesticides and pharmaceuticals quite generally, are discovered and developed in industrial laboratories, whereas more fundamental research on modes of action is usually conducted in governmental research centres.

For discovering new fungicides the empirical approach must still be used. The techniques involved in the evaluation of chemicals as fungicides have recently been reviewed by Torgeson.[1] There is a trend towards the elimination of *in vitro* methods and the use of practical fungitoxicity tests. As there is a possibility of specificity of fungicidal activity, it is necessary to come as close to the object as is permitted by suitability as a laboratory test; therefore a survey of frequently-used methods can give us an idea of the objectives of fungicide testing. The methods applied in fungicide screens in industry are not regarded as being secret, yet not many surveys of total screens have been published. Without any pretence of exhausting the subject I should like to show some tables representing a number of frequently-used methods.

Foliar protectants

The prime object of the testing of chemicals against powdery mildew is apple powdery mildew, because fruit fungicides are interesting from a commercial point of view; (Table 1); in the second place powdery mildew of cucurbits, cereals, vine and ornamentals is important. Almost every screen comprises at least one test against a powdery mildew, not necessarily because the market for mildewicidal compounds is more interesting than any other separate

*Introductory paper presented at the First International Congress of Plant Pathology, London 14–28 July 1968.

Table 1
Laboratory tests for foliar protectants

Fungus	Host
1 Powdery mildews	
Podosphaera leucotricha	apple
Sphaerotheca fuliginea	cucumber
Erysiphe graminis	wheat, barley
Erysiphe polygoni	bean
Uncinula necator	vine
2 Rusts	
Uromyces phaseoli	bean
Puccinia sp.	wheat
3 Others	
Phytophthora infestans	tomato
Peronospora tabacina	tobacco
Plasmopara viticola	vine
Alternaria solani	tomato
Botrytis cinerea	vine, lettuce
Botrytis fabae	broad bean
Venturia inaequalis	apple
Piricularia oryzae	rice
Colletotrichum lindemuthianum	bean
Colletotrichum lagenarium	cucumber
Septoria apii	celery
Cercospora beticola	beet

disease, but because the *Erysiphaceae* behave very exceptionally in their sensitivity to fungicides. Tests with rusts are, I think, more widely used in the USA and England than in Continental Europe, especially the bean rust test. It is questionable whether this popularity is in accordance with the commercial feasibility of developing a rust fungicide.

Of the third group, the tests with early and late blight are very popular, one of these being found in every screen, quite often in the primary screen, partly because of easy handling and partly because of the importance of these diseases. Tests with apple scab and vine mildew are very common in the secondary screens of European pesticides laboratories, but are only occasionally performed at test stations of the USA. Rice blast, lastly, perhaps the most important plant disease of the world, figures, of course, in every screen in Japan but is popular also in Europe and the USA.

Although the earlier remark on the frustrations encountered in developing modern commercial fungicides in the first place holds true for foliar protectants, testing for this type of chemical is still popular. Why? Just a question of conservatism of capitalistic chemical industries? Since a discussion on methods of testing for foliar protectants except against powdery mildew, is lacking in today's programme I may, perhaps, be allowed to volunteer a few more remarks on this subject.

Chemical industries are still testing for foliar protectants for the following reasons:

1 Although quite a lot of reasonably effective and cheap chemicals are available for use as foliar protectants, and a search for new ones does not seem of general interest, for an industrial firm the finding of new foliar protectants is of private interest. Market analysis shows us that the main portion of the fungicides market consists of foliar protectants, so it is logical for every firm to try and get a part of this market.

2 The testing of chemicals for foliar protection involves the possibility of finding eradicant types of fungicides which are clearly needed. A fungicide with curative properties can greatly reduce the risk of the grower, and will make elaborate protective spraying programmes less necessary.

3 There is also a possibility of finding certain types of systemic fungicides with these methods. I shall revert to the systemic fungicides later on. Furthermore, foliage fungicide tests are often used to investigate the weatherability of candidate fungicides and new formulations of existing fungicides; for this purpose delay periods between spraying and inoculation, and artificial rain are introduced in the test.

An illustration of the fact that the conservative approach of the search for protective activity gives a fair chance of discovering new fungicides, at least structure-activity relationships, is given in Table 2. In this table the results of screening a series of 1,500 chemicals in the fungicide laboratory of Philips-Duphar are presented. To make it less complicated I have included the results of only three of the seven objects used in our primary screen. In the first column the full data are given, in the second column three groups of related chemicals, synthesized to follow up earlier findings, have been left out, so that this column gives a more honest impression of the results of random screening of a group of chemicals which were admittedly not really chosen at random.

The number of chemicals active against spores of *F. culmorum* at 100 ppm in the slide germination test, is rather high (12 and 9 per cent, respectively) and the percentage effective as foliar protectants against *P. infestans* is somewhat lower (10 and 6 per cent), the correlation

Table 2
Results of the screening of 1,500 chemicals

Method	screening concentrations ppm	active out of the total group (1500)		3 groups of related chemicals left out (1430)	
1. *Fusarium culmorum*, slide germination	100	178	(12%)	131	(9%)
2. *P. infestans*, tomato leaves	300	153	(10%)	90	(6%)
3. Cucumber powdery mildew	300	26	(2%)	9	(1%)
active in 1 and 2 not in 3		103		76	
active in 1 and 3 not in 2		3		3	
active in 2 and 3 not in 1		0		0	
active in 1 and 2 and 3		18		2	
Exclusively active in 1		54		49	
Exclusively active in 2		50		30	
Exclusively active in 3		5		4	

between these two tests being fairly high but not complete; 76 + 2 were active in both; some chemicals are effective in the slide germination test, but not against *Phytophthora*, and for quite a large number the reverse is true. The occurrence of the latter type of chemicals illustrates the risk of relying solely on screening *in vitro*. The percentage of chemicals active against powdery mildew is very low, and the specificity of this type of activity is apparently rather high (4 of 9).

Seed protectants

Another object in fungicide testing is for seed treatment (Table 3). The costs of seed treatment in relation to potential gains are so low that it is currently regarded as an insurance, and consequently nearly all seed sown in the agriculturally advanced countries is treated. However, few primary screening programmes include tests for evaluation of chemicals for seed treatment. The effectiveness and cheapness of the materials now in use, on the one hand, and the difficulty of laboratory handling of the main objects such as smuts and bunts, on the other, are, no doubt, responsible for this state of affairs. So the tests mentioned in this table form part of secondary screening programmes, and activity against smuts and bunts is currently tested only once a year in the greenhouse or the field with a limited number of chemicals. The growing concern over the toxicity of organic mercurials and consequently the restrictive measures on their application announced or already taken in a number of countries, has caused a growing interest in this type of testing.

Table 3
Seed treatment: Laboratory techniques

Object	Fungus	Seed
Seed protection	Pythium and other fungi in soil	Pea, corn (cold testing)
Seed disinfection	*Septoria nodorum*	wheat
	Fusarium sp.	wheat
	Helminthosporium avenae	oats
	Phoma betae	beet

Table 4
Soil treatment: Laboratory techniques

fungus	indicator plants
Rhizoctonia solani	cotton, pea
Pythium ultimum	pea
Phytophthora parasitica	cucumber, citrus
Phytophthora nicotianae	tobacco
Fusarium oxysporum	tomato
Sclerotium rolfsii	pea
Sclerotinia minor	lettuce

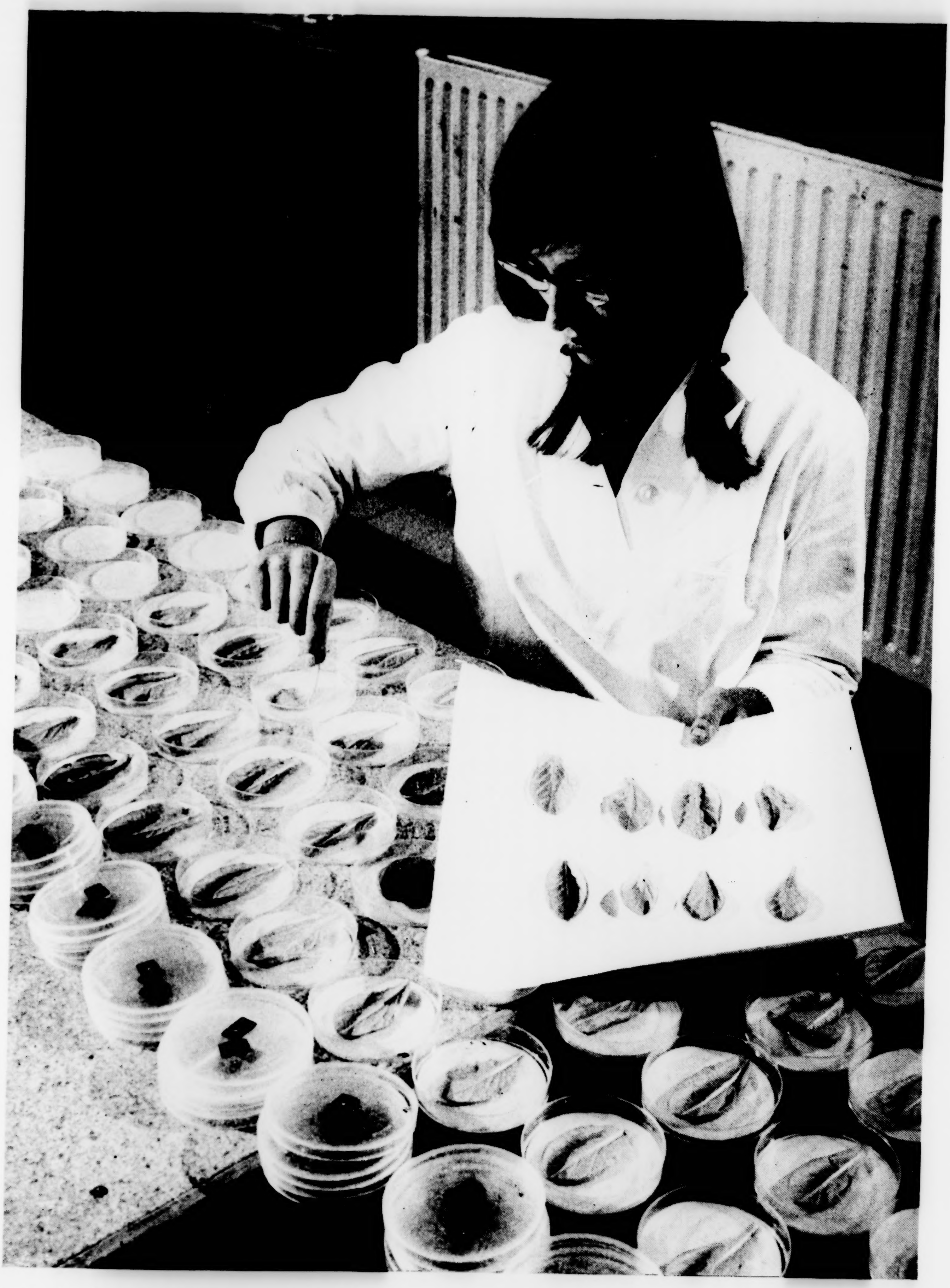


Fig. 1 A technician arranging detached lettuce leaves in petri dishes for testing chemicals on *Botrytis cinerea*

Fig. 2 Assessment of the infection of rice seedlings by *Piricularia oryzae*.
The plants are incubated in moist chambers under controlled temperature and light conditions

Soil fungicides

Soil mix tests and drench tests for evaluating soil fungicides are included in primary screening programmes more frequently than seed treatment tests; (Table 4). The potential market is undoubtedly enormous. Another reason for their popularity may be the possibility of coordinating the soil fungicide test with soil herbicide and soil nematicide tests. And does not fashion play a role in the prevailing opinion which regards testing for soil fungicides as a more sophisticated approach than testing for seed disinfectants? At any rate, taking into account the high costs for controlling a disease by application of soil fungicides, some scepticism seems to be justified. The tests are performed either with or without indicator plants for assessment of fungicidal activity. *Rhizoctonia solani* is undoubtedly the most popular test organism in industrial screening programmes.

Systemic fungicides

With Table 5 we will pass on to the subject of systemic fungicides. That a part of the work of screening for systemics is aimed at vascular diseases needs no further comment. For testing systemics against foliar diseases, the methods used for testing foliage fungicides, as mentioned in Table 1, can readily be adapted simply by modification of the site of application. In the table only some popular tests are mentioned. Although a special section of this symposium will be devoted to systemic fungicides, I venture upon making a few remarks because I find it difficult to ignore this subject altogether in a discussion on the objectives of fungicide testing.

Table 5
Laboratory and greenhouse tests for systemics

Test organism	Host
Wilt diseases	
Fusarium oxysporum	tomato, lupinus
Foliar diseases	
Uromyces phaseoli	bean
Sphaerotheca fuliginea	cucumber
Cladosporium cucumerinum	cucumber
Botrytis fabae	broad bean
Phytophthora infestans	tomato

The ideal 'fungicide' can be regarded as a compound which by one single application, for instance as a seed dressing, protects all parts of the emerging plant and continues doing so during its whole life without being toxic or even fungicidal, but merely by supporting the plant in its natural resistance reactions, for instance by interference with the host–parasite relationship. We phytopathologists feel a need for a more sophisticated approach towards the realization of this ideal. The search for systemic fungicides might be regarded as just a first step in this direction. On the occasion of the 50th anniversary of the American Phytopathological Society Dr Dimond stated 'We have spent too much time in looking for ever better sprays and have spent too little effort in searching for compounds that are moved through the plant and lie in wait for the pathogen as it enters'.

Ten years have since passed and what about the present situation? Professor McCallan in his excellent survey on 'History of fungicides' in Torgeson's book observes the following 'while there is yet no outstanding commercial control of plant diseases by systemic fungicides, interest continues and the practical solution is probably only a matter of time.' And although recent findings are indeed very promising for the near future, at present, in common practice the main role is still played by old fashioned foliar protectants and seed disinfectants.

The following reasons may be mentioned for this rather slow development: In the first place, most chemicals entering a plant are liable to cause phytotoxic symptoms. In the second place, the presence of a chemical within the plant, especially in the case of edible crops, may be rather a nuisance with residue problems. In the third place, the physicochemical criteria of uptake into and translocation within plants seem to be contradictory to those of fungitoxicity: for translocation polarity is required, whereas for fungicidal activity apolar compounds are in favour. In order to overcome this paradox we have to move from the classical type of fungicide, which achieves its selective toxicity by selective accumulation in the fungal cell, to a type which achieves this either by selective interference with vital processes of the fungus or by interacting with the host–parasite relationship. The chance of finding such compounds is of course fairly poor whereas the more classical type is obviously far more abundant. This leads to a fourth factor, a psychological one: The industrial research worker dealing with elaborate screening programmes of thousands of chemicals a year, like every other research worker, wants to see results, to discover relationships. But his imagination is not stimulated by screening thousands of chemicals for systemic activity without seeing

any result. Therefore more basic research is needed, which cannot be afforded by industrial research groups alone. Here I think much more help is needed from our universities and governmental institutes.

Conclusions

In this context let me make this point: there is a general demand for highly selective pesticides. However, in respect of control of fungi we should not go too far in this direction: In the first place with highly selective fungicides the chance of inducing the fungus to develop resistant races is not altogether negligible. In the second place it is not attractive from a practical point of view, neither for the farmer who has to deal with quite a number of diseases nor for the industrial firm for which it is economically prohibitive to develop a fungicide for a limited market. I think, especially for difficult problems of only local interest or limited economical importance, the attention of governmental research groups should be asked. The main objective of testing fungicides is the discovery of compounds that have to be applied less frequently and in smaller amounts than present-day fungicides. This is both of private interest to the industrial firm discovering such a chemical and of general public interest. For a more sophisticated approach of disease control a coordinated effort of governmental and industrial research workers is essential.

References

1 Torgeson, Dewayne C., 1967. '*Fungicides. An Advanced Treatise.*' Academic Press, New York and London.

2 McGrath, H., 1964. Chemicals-plant disease control. *Agric. Chem.* **19**, No. 7 (18–22); No. 8 (22–27); No. 9 (28–37).

3 Wellman, R. H., 1967. Commercial fungicide development. In '*Fungicides, an Advanced Treatise*' (D. C. Torgeson, Ed.) p. 128.

Sur les Végétaux parasites (non-microbiens) transmissibles des Animaux a l'Homme, et réciproquement.

PAR

le Dr. RAPHAËL BLANCHARD, Professeur agrégé à la Faculté de Médecine de Paris, Secrétaire Général de la Société Zoologique de France.

Parmi les champignons parasites de l'homme, il en est quatre seulement dont on puisse démontrer sûrement la transmission directe à notre espèce par les animaux avec lesquels nous sommes ordinairement en rapport. Ce sont :—

Achorion Quinckeanum, Zopf, 1890;
Achorion Schoenleini, Remak, 1848;
Trichophyton epilans, Mégnin, 1878;
Trichophyton tonsurans, Malmsten, 1848.

La contagion directe des animaux à l'homme est très probable, mais insuffisamment démontrée pour les quatre microphytes suivants :—

Actinomyces bovis, Harz, 1877;
Microsporon Audouini, Gruby, 1843;
Lepocolla repens, Eklund, 1883;
Aspergillus fumigatus, Fresenius;

Nous allons résumer brièvement les faits qui démontrent l'exactitude des prémisses ci-dessus énoncées.

ACHORION QUINCKEANUM, Zopf, 1890.

Synonymie: *Achorion Arloingi*, Busquet, 1891.

Ce champignon est la cause de la teigne faveuse de la souris. Dès 1854, Draper, médecin à New York, indiquait la transmission du favus de la souris au chat, puis de celui-ci à l'homme. Depuis lors, le professeur Saint-Cyr, de l'Ecole vétérinaire de Lyon, a apporté de nombreuses preuves à l'appui de cette même idée. C'est d'ailleurs l'Ecole dermatologique de Lyon qui, par une remarquable série d'observations, a mis hors de doute ce fait actuellement admis par tous les médecins, qu'une certaine forme de teigne faveuse nous est transmise par les petits rongeurs. La spécificité de ce favus est démontrée par deux ordres de phénomènes :—

1°. Ensemencé sur la gélatine ou dans différents milieux nutritifs, l'*Achorion Quinckeanum* donne une culture luxuriante, très différente de celle qu'on obtient avec le favus ordinaire de l'homme. C'est lui que Quincke a désigné sous le nom de microphyte α et que Bœr en 1887 et Busquet en 1890 ont retourné par la culture directe du favus de la souris.

2°. Au point de vue clinique, le favus provenant de la souris se distingue par des caractères très spéciaux, notamment par la rareté des godets faviques. Quincke désigne cette variété de teigne faveuse sous le nom de *Favus vulgaris*.

Le premier cas de favus chez les animaux a été observé par Jaquetant, en 1847: deux chats de l'Antiquaille, à Lyon, étaient devenus faveux au contact de deux fillettes qui avaient l'habitude de jouer avec eux.

En 1877, Saint-Cyr a vu plusieurs élèves de l'Ecole vétérinaire de Lyon qui étaient atteints de favus: dans le placard renfermant leur linge de corps, on trouva des souris faveuses, qui avaient sans doute eté le point de départ de la contagion. Tripier s'est inoculé avec succès le favus de la souris.

D'autre part, des inoculations positives de favus ont été faites de l'homme au chat par Saint-Cyr et Vincens, de l'homme à la souris par Tripier et Vincens, et de l'homme au rat (1) par Gigard. Deux tentatives d'inoculations de l'homme au chien, faites pas Vincens, n'ont donné aucun résultat. Rien ne prouve, dans aucun de ces cas, que le microphyte transmis ait été l'*Achorion Quinckeannm* plutôt que l'*Achorion Schoenleini.*

ACHORION SCHOENLEINI, Remak.

Ce champignon correspond aux formes β et γ distinguées par Quincke; c'est lui seul que Fabry et Elsenberg ont obtenu dans leurs cultures. Il détermine cette variété de teigne faveuse que Quincke désigne sous le nom de *Favus herpeticus* et qui peut d'ailleurs s'observer parfois chez un malade, en même temps que le *Favus vulgaris.*

On admet généralement que le favus se communique assez rarement de l'homme à l'homme; Alibert niait même sa contagion, mais Jaquetant, Remak et Deffis ont prouvé par des expériences rigoureuses la réalité de celle-ci. Lailler a traité à l'hôpital Saint-Louis un malade qui avait contracté la maladie en couchant dans un lit occupé précédemment par une personne atteinte de favus.

On a cru longtemps que le favus était ordinairement transmis à l'homme par le chat, contaminé lui-même préalablement par le rat ou la souris. La distinction récemment établie entre l'*Achorion Quinckeanum* et l'*Achorion Schoenleini* démontre que le *Favus herpeticus* ne nous est pas transmis par le chat, voire même par le chien, ou par les petits rongeurs, mais rend d'autre part très incertaine la provenance de cette dermatose. Assurément, la possibilité de la contagion réciproque dans l'espèce humaine rend compte de certains cas, mais ne saurait les expliquer tous. Il est probable que l'homme peut s'infester au contact de certains animaux, mais on ne saurait dire actuellement quels animaux doivent être incriminés.

Le chien est parfois atteint de teigne faveuse, mais les résultats positifs obtenus par Saint-Cyr en inoculant à cet animal le favus du chat, tendent à faire admettre qu'il s'agit habituellement de la teigne

---

(1) Dans le *Roman du Renard,* qui date du XIe siècle, le rat est appelé *Pelé.* Ce nom vient-il de ce que la queue du rat est à peu près glabre, ou n'est-il pas dû plutôt à ce que, à cette époque, le rat (*Mus rattus*) était déjà frèquemment atteint de teigne faveuse?

causée par l'*Achorion Quinckeanum*. Par ses rapports journaliers avec le chat familier, le chien doit d'ailleurs se contaminer assez aisément. Le chien contracte aussi sans difficulté la teigne du lapin, d'après d'autres expériences de Saint-Cyr.

Le cheval et le bœuf peuvent également être atteints de favus, encore que ce soit là une manifestation morbide assez rare. En 1880, Gigard a observé une épidémie de teigne faveuse qui sévissait tout-à-la fois dans l'espèce bovine et chez les enfants : dans ce cas, la transmission de l'animal à l'enfant ne semblait pas douteuse. Il y a là une indication précieuse, au point de vue de l'étiologie du favus humain.

Ercolani décrit en 1876, sous le nom d'*Achorion keratophagum*, un microphyte qu'il a rencontré dans le sabot des solipèdes atteints de fourmilière et qu'il considère comme la cause de cette maladie. En raison de l'analogie de celle-ci avec la rogne ou carie sèche des ongles humains, Ercolani admet encore que la rogne est causée par le même parasite. Cette opinion ne repose d'ailleurs sur aucune observation positive, et la spécificité de l'*Achorion keratophagum* n'est pas plus démontrée au point de vue botanique qu'au point de vue clinique.

En 1858, Müller, Gerlach et d'autres ont observé chez le coq et la poule un favus de la crête et des caroncules. En 1881, Mégnin étudie avec soin le champignon qui cause cette affection et lui donne le nom d'*Epidermophyton gallinæ*, le considérant comme spécifiquement distinct de l'*Achorion Schoenleini*. Les parties malades présentent des croûtes blanches, farineuses ou plâtreuses ; les godets faviques font défaut. Le microphyte est caractérisé par un mycélium fin, court, tortueux, émettant des sporophores cloisonnés, terminés par des chapelets de 5 à 6 spores rondes, larges de 6 à 8 μ, plus volumineuses que celles de tous les autres champignons parasites de nos animaux domestiques. Il pullule entre les lames épidermiques, sans s'introduire dans les follicules plumeux. Il se cultive bien sur la gélatine ; il s'y développe à la surface en touffes d'un blanc de neige, tandis que la gélatine se liquéfie et prend la couleur du jus de groseille.

Au contraire, Neumann (de Toulouse) assure que le favus des poules est dû simplement à l'*Achorion Schoenleini*. Il base son opinion sur la ressemblance morphologique des deux champignons et sur le résultat de quelques inoculations. Chez un jeune chien, inoculé avec le favus de la poule, il se développe une dermatose identique à celle qu'on obtient chez un autre chien, après inoculation du favus de l'homme. Cette même expérience comparative réussit sur deux lapins, sans que rien permette de soupçonner l'origine différente des deux affections. Inversement, on reproduit le favus de la poule en déposant sur la crête des croûtes de favus humain délayées dans l'eau. De ces expériences il semble donc résulter que la poule doit être envisagée comme l'une des sources du favus humain.

Trichophyton Depilans, Mégnin, 1878.

Ce microphyte est la cause de la teigne tonsurante dans l'espèce bovine. Ernst, médecin dans le canton de Zurich, signalait dès 1820 que l'herpès tonsurant peut être communiqué à l'homme par les

animaux : une jeune fille avait manifestement contracté la maladie en trayant une vache qui en était elle-même atteinte. Des faits de ce genre ont été signalés à l'attention des médecins par Grognier en 1831, Kollreuter en 1836, Lavergne et Fehr en 1838, Epple en 1839, Rademacher en 1842, Houlez (de Sorèze) en 1845, Horing en 1846, Letenneur et Malherbe (de Nantes) en 1851, puis par Reynal en 1858.

La maladie acquise ainsi par l'homme est un herpès circiné qui diffère notablement de l'herpès tonsurant ordinaire. Mégnin a complété ces démonstrations en mettent en relief, en 1878, les différences essentielles, tant morphologiques que cliniques, qui caractérisent le *Trichophyton depilans.*

A l'aide d'expériences directes, Gerlach a démontré que la teigne tondante est transmissible du bœuf au bœuf, du bœuf au cheval, et du bœuf au chien ; toutefois, chez ce dernier, l'inoculation réussit assez difficilement. Gerlach n'a jamais eu qu'un résultat négatif en cherchant à contaminer le mouton et le porc par le bœuf, mais Perroncito a observé la transmission de la teigne du bœuf à l'agneau.

TRICHOPHYTON TONSURANS, Malmstén, 1848.

En 1853, Bazin a publié la fameuse observation de gendarmes qui avaient contracté la teigne tondante en soignant des chevaux atteints de "dartre." Galligo en 1858, Horand (de Lyon) en 1871, Dieu en 1876, Larger en 1881, et Longuet en 1882 ont publié des cas analogues.

En 1881, Mégnin a observé une quinzaine d'artilleurs d'une même batterie, qui présentaient des cercles d'herpès circiné sur le cou et le menton. Etant au camp pour les manœuvres, ils avaient pris les couvertures des leurs chevaux pour se préserver du froid et s'y étaient enveloppés jusqu'au menton. Or, ces chevaux étaient récemment arrivés des dépôts de remonte et étaient atteints de dartre tonsurante, comme un examen attentif permit de le constater.

Le cheval peut donc transmettre à l'homme la teigne tonsurante. Cette transmission s'observe même assez fréquemment dans les régiments de cavalerie. En France, la maladie est importée par de jeunes chevaux venus des haras de Normandie, pays où la teigne tonsurante existe à l'état endémique chez les animaux d'espèce bovine. Si, comme on l'a dit, les poulains se contaminent dans les pâturages, au contact des bœufs et des veaux, ou bien la teigne tondante du cheval pourrait reconnaître une double origine (*Trichophyton depilans* et *Tr. tonsurans,* suivant les cas), ou bien le *Trichophyton depilans* serait une simple variété du *Trichophyton tonsurans.* C'est là une question importante, sur laquelle il est difficile de se prononcer actuellement.

La teigne tonsurante peut aussi nous être communiquée par le chien ; les cas étudiés par Purser en 1865 et par Horand en 1872 et en 1873 en donnent une preuve convaincante. L'observation de Purser et la première observation de Horand sont particulièrement remarquables, en ce qu'elles nous montrent que l'onychomycose ou trichophytie unguéale peut provenir du chien.

Le chat lui-même peut devenir un agent de transmission. Tuckwell en 1871, Lancereaux et Michelson en 1874, ont publié des observations qui ne laissent aucun doute à cet égard.

Quant à la transmission de la teigne tondante des ruminants à l'homme, on doit faire des réserves expresses, car nous pensons que, dans la majorité des cas, sinon dans tous, la contagion s'est faite par l'intermédiaire du *Tricophyton depilans*.

Toutefois, nous ne pouvons affirmer que ce champignon soit la cause exclusive de la trichophytie bovine, puisque le *Trichophyton tonsurans* s'inocule très facilement au veau. La teigne tondante de l'homme se transmet aussi très aisément au chien (Cramoisy, 1856; Vincens, 1874) et au chat (Vincens), mais ne se transmet pas aux rongeurs (souris, rat, lapin). Ajoutons que cette même dermatose se propage tout aussi bien entre animaux d'espèce différente, par exemple du chat au cheval (Williams).

Mégnin a décrit chez le lapin, sous le nom de *Teigne lycoperdoïde*, une maladie que certains auteurs considérent comme identique à la trichophytie tonsurante, mais qui semble bien en être distincte, d'abord à cause de ses caractères cliniques très spéciaux, puis à cause de la difficulté (pour ne pas dire l'impossibilité) que l'on éprouve à transmettre au lapin la teigne tondante de l'homme.

Actinomyces Bovis, Harz, 1877.

Ce champignon ne saurait rentrer dans le genre *Actinomyces*, Meyen, 1827; ce serait donc un acte de justice que de le désigner sous le nom de *Discomyces*, proposé par Rivolta. La maladie qu'il détermine et qui porte le nom d'*actinomycose*, prendrait alors celui de *discomycose*.

En raison même du titre de ce rapport, il peut paraître hors de propos de mentionner ici l'*Actinomyces*, que certains auteurs rangent parmi les Desmobactériacées, à côté du *Cladothrix dichotoma*. Nous ne méconnaissons point la valeur des raisons invoquées en faveur de cette opinion. Si pourtant nous continuons à rattacher l'*Actinomyces* aux champignons proprement dits, c'est uniquement parce que ses caractères morphologiques ne nous semblent pas suffisamment établis pour qu'on puisse lui attribuer une place certaine et définitive dans la classification.

L'actinomycose n'a encore été observée que chez l'homme, le bœuf, le cheval et le porc; chez les trois premiers, elle siège dans des organes très différents, mais surtout au voisinage du tube digestif ou dans le poumon, d'où l'on peut conclure que le champignon pathogène s'introduit dans l'organisme soit avec les aliments, soit avec l'air inspiré. Chez le porc, on ne le trouve que dans les muscles. En somme, et c'est là un fait capital, l'actinomycose n'est pas une maladie de la peau ou des muqueuses, ce qui constitue déjà un premier argument contre la nature contagieuse de cette affection.

Elle ne semble pas davantage être transmissible par l'usage de la viande malade. D'ailleurs les carnivores, non seulement ne contractent pas spontanément la maladie, mais encore ne la prennent pas par

inoculation directe des cultures pures d'*Actinomyces*; en revanche, l'inoculation réussit très bien chez le lapin.

La contagiosité de l'actinomycose est admise par divers auteurs : Hacker, Stelzner et Israël ont cité des cas où des personnes auraient été contaminées parce qu'elles se trouvaient en contact avec des animaux malades. En 1888, Boracz a publié l'observation d'un cocher qui, sans jamais avoir été en rapport avec des animaux malades, était pourtant atteint d'actinomycose du maxillaire; quatre mois plus tard, sa femme présentait elle-même tous les signes de la maladie.

En se basant sur ces observations, on admet la contagion de l'animal à l'homme et même de l'homme à l'homme. Cette conclusion est-elle rigoureuse ? Nous ne le croyons pas.

Les observations ci-dessus démontrent simplement que les individus qui en sont l'objet se sont trouvés dans les conditions mêmes dans lesquelles le bétail contracte la maladie. Or, ces conditions sont actuellement connues : on soit que l'*Actinomyces* se trouve répandu sur les graminées et qu'il est introduit dans le poumon par la poussière émanant de celles-ci ou sous la peau et les muqueuses par les barbes de céréales. N'est-il pas vraisemblable que les deux individus dont il est question dans l'observation de Boracz ont pu être contagionnés de cette manière ? Et la même explication n'est-elle pas également valable pour les bouviers, gens de ferme, etc., qui contractent la maladie en même temps que le bétail et dans des conditions sensiblement identiques ?

Nous trouvons un dernier argument contre la contagiosité dans les statistiques publiées par divers auteurs, notamment par Moosbrügger, qui a relevé jusqu'à 75 cas. Sur ce nombre, 10 cas seulement se rapportaient à des propriétaires fonciers, à des paysans, à des valets de ferme; tous les autres cas s'observaient chez des personnes appartenant aux professions les plus diverses et pour lesquelles la contagion directe ne saurait être admise.(1)

MICROSPORON AUDOUINI, Gruby, 1843.

Synonymie : *Microsporon decalvans*, Bazin, 1853. *Trichophyton decalvans*, Bazin, 1873.

On peut distinguer trois formes de pelade :—

1°. Une pelade non parasitaire et non contagieuse, due vraisemblablement à une trophonévrose.

2°. Une pelade parasitaire, due à un *Micrococcus* qui envahit le follicule pileux. Cette pelade, entrevue par Thin en 1881 et par von Sehlen en 1883, a été bien étudiée récemment par H. Nimier, puis par L. Vaillard et H. Vincent ; elle est probablement contagieuse, mais rien ne permet encore de supposer qu'elle ait le moindre rapport avec une maladie quelconque des animaux.

---

(1) Au moment où nous corrigeons les épreuves de cet article, nous pouvons prendre connaissance des comptes-rendus sommaires du Congrès d'hygiène, publiés par les journaux de médecine. Nous y voyons que M. Crookshank (de Londres) et M. le professeur Nocard (d'Alfort) ont soutenu, relativement à l'origine de l'actinomycose, une opinion toute semblable à celle que nous exposons plus haut.

3°. Une pelade parasitaire, due au *Microsporon Audouini* et se présentant sous deux aspects : pelade achromateuse et pelade décalvante. Bazin considérait cette dernière comme une entité morbide distincte et lui attribuait un microphyte spécifique.

La pelade par *Microsporon,* la seule qui doive nous arrêter, est transmissible de l'homme à l'homme : le fait est suffisamment démontré pour qu'il soit inutile d'en donner des preuves nouvelles. En revanche, la transmission à l'homme de l'alopécie des animaux, bien que vraisemblabe, n'est nullement certaine.

Dès 1856, Rivolta [1] a observé un bœuf qui portait sur le ventre une plaque d'alopécie : "peu-à-peu elle s'étendit et devint presque "générale ; tous les poils tombèrent et la peau resta d'une couleur "obscure brillante. Les paysans croyaient cette maladie contagieuse et "la craignaient." En 1874, Hillairet a publié la curieuse observation de six employés du chemin de fer de l'Est, à Paris, tous atteints de pelade et auxquels, selon toute vraisemblance, la maladie avait été communiquée par un chat. Arnozan (de Bordeaux) a fait connaître en 1885 une série de cinq observations de pelade chez des personnes qui avaient été probablement contaminées par des animaux (chien et chat) atteints eux-mêmes d'alopécie ; la recherche du *Microsporon* spécifique n'a été fait dans aucun de ces cas.

Il est certain du moins que la pelade est fréquente chez le jeune chat, comme l'a fait remarquer Baillet, et que sa propagation de chat à chat est incontestable. L'alopécie circonscrite est également assez commune chez le chien (Siedamgrotzky), chez le veau (Perroncito) et se voit même à la racine de la queue du cheval ; dans ce dernier cas, suivant Leisering, l'"*herpes caudalis*" est caractérisé par la présence de champignons.

Si des observations cliniques ultérieures viennent confirmer les faits qui précèdent ; si d'autre part des inoculations de l'animal à l'homme et de l'homme à l'animal démontrent la transmissibilité de la pelade ; si enfin les cultures des champignons recueillis tant sur l'homme que chez les animaux se montrent toujours identiques, l'exactitude des prévisions que nous venons d'énoncer sera définitivement établie. A ces conditions seulement il sera possible d'affirmer que la pelade de l'homme et l'alopécie des animaux sont une seule et même maladie. En attendant cette démonstration rigoureuse, il sera prudent de tenir à l'écart et de traiter par les procédés antiparasitaires tout animal dont les poils tombent par plaques.

Lepocolla repens, Eklund, 1883.

Synonymie : *Epidermophyton,* Lang, 1879 (nec Mégnin, 1881).

Pour certains auteurs, le psoriasis est une affection d'origine purement nerveuse, non parasitaire et non contagieuse ; pour d'autres, c'est, au contraire, une dermatomycose.

---

[1] *Dei parassiti vegetali,* p. 469. Torino, 1873.

Dès 1878, Lang (d'Insbrück) a soutenu cettee dernière opinion. Dans les squames psoriasiques, il trouve un champignon qu'il désigne sous le nom d'*Epidermophyton* et dont la présence est constante : s'il est resté jusqu'alors inaperçu, cela s'explique bien plus par la pâleur de ses filaments mycéliens et de ses spores que par leur exigüité. Balzer (de Paris) en 1881 et Eklund (de Stockholm) en 1883 retrouvent le même parasite.

Ce dernier observateur croit reconnaître que la maladie débute par les capillaires des papilles dermiques : le mycélium se développe autour de ces capillaires, puis envahit la profondeur de la couche muqueuse de Malpighi, dont les cellules se montrent bientôt enserrées dans un lacis de filaments mycéliens, portant des spores en massue. Les écailles de psoriasis, ensemencées dans des milieux convenables, donnent un abondant mycélium, dans les filaments duquel se forment des spores endogènes. La démonstration eût été convaincante, si la maladie avait été reproduite expérimentalement, par inoculation des cultures ; mais cette recherche n'a pas été faite.

Wolff (de Strasbourg) a observé, lui aussi, le *Lepocolla ;* les spores siégeraient de préférence dans les parties les plus profondes de l'épiderme.

Quelques auteurs, tout en admettant la nature parasitaire du psoriasis, ne croient pas à la spécificité du *Lepocolla :* telle est du moins l'opinion d'Unna et de Quinquaud. Sans se prononcer sur la question, Mapother déclare simplement que l'agent pathogène est un organisme anaérobie, qui ne vient pas directement du dehors, mais envahit plutôt la peau par les capillaires du derme, qu'il obstrue et qu'il finit par déchirer.

En somme, la théorie parasitaire du psoriasis compte des partisans sérieux et convaincus.

La contagion de la maladie dans l'espèce humaine a d'ailleurs été mise en évidence par Unna (de Hambourg), qui vit une domestique psoriasique, récemment admise dans une famille, transmettre le psoriasis à trois enfants confiés à sa garde. Aubert (de Lyon) a vu, de son côté, un mari transmettre la maladie à sa femme. Enfin, Augagneur (de Lyon) a observé à l'Antiquaille un teinturier chez lequel se déclara un psoriasis progressivement généralisé, au bout de quatre mois de séjour à l'hôpital, entre deux psoriasiques ; l'intérêt très spécial de cette observation réside en ce que ce malade était primitivement atteint d'un eczéma professionnel, qui se transforma directement en psoriasis.

D'après ces faits, on peut donc penser que les cas que, jusqu'à présent, on a cru pouvoir attribuer à l'hérédité, s'expliquent plus justement par une transmission direction des parents aux enfants.

Le psoriasis humain est contagieux pour les animaux. A la suite de frictions répétées avec des squames recueillies sur un malade, Lassar a pu inoculer trois lapins. Des expériences analogues, variées de diverses façons, ont été faites par Tommasoli et par Beissel. Non seulement la maladie se développe chez le lapin, quand on frictionne la peau de ce rongeur, mais même quand on lui injecte sous la peau, dans

le péritoine ou dans la veine jugulaire, des squames psoriasiques délayées dans une solution faible de chlorure de sodium. Bien plus, le psoriasis communiqué expérimentalement par le lapin est transmissible à un autre lapin, auquel cas la période d'incubation est remarquablement courte.

A côté de ce psoriasis expérimental et artificiel, il convient de rappeler qu'une affection psoriasique, peut-être identique au psoriasis humain, s'observe chez le cheval, l'âne et le mulet, ce qui, selon la juste remarque d'Eklund, explique pourquoi les cochers sont assez fréquemment atteints par la maladie.

Un psoriasis inoculable à l'homme frappe également l'espèce bovine, et c'est là, pensons-nous, un fait d'une importance capitale, car une étude attentive pourrait montrer la fréquence des faits dont nous allons parler et jeter une vive lumière sur l'étiologie toujours obscure du psoriasis humain.

En 1887, Tenholt constata que des bœufs d'origine hollandaise étaient atteints d'une maladie cutanée qui avait la plus grande ressemblance avec le psoriasis. Elle se communiquait de l'animal à l'animal et passa aussi sur quatre individus chargés de garder et de soigner les animaux malades. Des cultures pures et l'inoculation de celles-ci sur des bœufs sains ne donnèrent aucun résultat positif. Il semble néanmoins démontré que l'homme puisse acquérir le psoriasis au contact du bœuf.

L'origine bovine de la maladie est, d'autre part, mise hors de doute par une importante série d'observations dans lesquelles on a vu nettement la maladie débuter, chez l'enfant et chez l'adulte, au niveau d'une pustule vaccinale obtenue avec le vaccin de génisse. Les premiers cas de ce genre ont été constatés aux Etats-Unis, en 1883, par Piffard, Th. Wood, Biart et Rohé; des observations identiques ont bientôt été faites en France, à Lyon, par Chambard, puis par Augagneur.

Aspergillus fumigatus, Fresenius.

Dieulafoy, Chantemesse et Widal (1) ont reconnu, en 1890, chez les pigeons vendus sur les marchés de Paris, une affection qui détermine, à la surface de la muqueuse buccale et dans le poumon, des lésions tout à fait comparables à celles de la tuberculose bacillaire, mais qui pourtant n'est point due au bacille de Koch. Les tumeurs contiennent à leur centre un mycélium qui, cultivé par les méthodes usuelles, acquiert tous les caractères de l'*Aspergillus fumigatus.* L'inoculation des spores à des pigeons détermine plus ou moins rapidement des lésions pseudo-tuberculeuses identiques à celles qui se développent spontanément chez ces animaux.

Bien qu'aucune autopsie ne soit encore venue en donner une preuve indiscutable, on peut affirmer que la même pseudo-tuberculose s'observe à Paris, chez les gaveurs de pigeons. Leurs expectorations ne renferment pas de bacilles, mais bien des fragments mycéliens. L'inoculation

---

(1) Dieulafoy, Chantemesse et Widal, *Une pseudo-tuberculose mycosique* Gazette des hôpitaux LXIII., No. 89, p. 821, 1890.

d'un crachat de malade à un pigeon produit chez ce dernier une tuberculose mycosique due à l'*Aspergillus fumigatus ;* l'ensemencement des crachats sur la gélose donne des colonies de ce même *Aspergillus*.

Voilà donc une aspergillose qui s'observe tout à la fois chez l'homme et chez l'oiseau. Existe-t-il une relation entre ces deux êtres, au point de vue de la propagation de la maladie ?

Pour pratiquer le gavage, le gaveur se remplit la bouche d'un mélange d'eau et de graines, puis ouvrant le bec du pigeon, il y applique ses lèvres et chasse par expiration une partie du mélange. Il est vraisemblable que le gaveur prend l'*Aspergillus*, cause de la pseudo-tuberculose pulmonaire, soit à la surface des graines qu'il introduit dans sa bouche, soit au contact direct de la tumeur buccale du pigeon. Il est même possible que celui-ci soit contaminé par l'homme qui, en soufflant pour projeter les graines, introduit dans les voies aériennes de l'oiseau quelques germes d'*Aspergillus*.

Microphytes insuffisamment connus.

En outre des champignons étudiés ci-dessus et dont les rapports avec les maladies contagieuses sont définis plus ou moins nettement, on connaît chez les vertébrés allantoïdiens un certain nombre de microphytes qui causent ou du moins accompagnent des maladies cutanées, mais dont on ignore encore l'origine ou les relations avec les maladies des autres animaux. Nous croyons utile d'en dresser la liste et d'indiquer très succinctement les affections dans lesquelles on les observe et les lacunes que présente leur histoire, dans le but d'attirer sur eux l'attention et de susciter des recherches qui puissent nous renseigner exactement sur leur valeur pathogénique, sur leur provenance et sur le degré de leur transmissibilité.

1°. *Selenosporium cuticola*, R. Bl., 1891.—Nous désignerons désormais sous ce nom un champignon dont nous avons fait un étude détaillée et qui cause, chez le lézard vert, une remarquable dermatose, probablement contagieuse pour les sauriens.

2°. *Microsporon pterophyton*, Mégnin, 1878.—Sur deux cacatoès qui perdaient leur plumage, Mégnin a reconnu que les plumes " étaient " envahies par un *Microsporon* très petit, englobant les barbules d'un " véritable feutre. Le mycélium est très distinct. Les sporules ont 1 à " 2μ." Mégnin ne dit pas avoir constaté la présence du microphyte dans la peau ; il n'est donc pas certain que celui-ci soit la cause de la chute des plumes. D'autre part, l'existence d'un mycélium donne à penser qu'il n'appartient pas au genre *Microsporon*.

3°. *Botryomyces*.—Ce champignon a des affinités manifestes avec l'*Actinomyces*. Signalé d'abord chez le cheval, il a été vu par Csokor (de Vienne) sur la mamelle de la vache. On ne sait rien de sa provenance ni de sa contagiosité.

Les champignons suivants sont particuliers à l'espèce humaine :—

4°. *Chioniphe Carteri*.—Il a été observé dans les cas de mycétôme ou pied de Madura. Sous ce nom, les auteurs ont décrit des maladies évidemment très diverses ; certaines observations semblent se rapporter simplement à l'actinomycose.

5°. *Oïdium albicans*, Ch. Robin.—Ce champignon est bien connu au point de vue clinique ; Linossier et Roux (de Lyon) l'ont étudié avec le plus grand soin au point de vue biologique. Néanmoins, on ignore encore s'il provient exclusivement de germes propagés par l'air ou s'il ne serait pas, dans certains cas, amené dans la bouche des enfants avec le lait de vache.

6°. *Oïdium lactis*.—On l'a vu dans le lait de brebis ; peut-être provoque-t-il des accidents chez de jeunes nourrissons ou chez de jeunes animaux nourris avec du lait. Ses relations avec l'*Oïdium albicans* méritent d'être déterminées avec plus de précision.

7°. *Microsporon anomæon*, Vidal, 1883.—Sous le nom de *Microsporon anomæon* ou *dispar*, le savant médecin de l'hôpital Saint-Louis a fait connaître un microphyte de l'épiderme, qu'il considère comme la cause du pityriasis circiné et marginé. En 1887, Mannino croit retrouver ce même parasite dans la séborrhée et lui conteste tout rôle pathogénique et spécifique ; nous pensons que le médecin italien a plutôt eu affaire au *Microsporon ovale*.

8°. *Microsporon furfur*, Ch. Robin, 1853 (*Malassezia furfur*, H. Baillon, 1889).—Le pityriasis versicolor est contagieux dans l'espèce humaine ; on ne sait rien de ses relations avec les maladies analogues chez les animaux.

9°. *Microsporon minutissimum*, von Bärensprung, 1862 (*Microsporon gracile*, Balzer, 1883).—Il cause l'érythrasma, affection dans laquelle Burchardt l'a découvert en 1859.

10°. *Microsporon ovale*, Bizzozero, 1884 (*Saccharomyces ovalis*, Bizzozero, 1884 ; *S. sphaericus*, Bizzozero, 1884, nec Nägeli, 1879 ; *S. capillitii*, Oudemans et Pekelharing, 1885 ; *Cercosphaera capillitii*, Oudemans et Pekelharing ; *Microsporon Malassezi*, H. Baillon, 1889).—Nous croyons pouvoir établir la synonymie ci-dessus pour l'organisme que Malassez (de Paris) a découvert en 1874 dans le pityriasis simplex.

11°. *Microsporon trachomatosum*, Noiszewski, 1890.—Sous le nom de *Microsporon trachomatosum sive jagium*, Noiszewski décrit un microphyte auquel il attribue la production du trachôme ou conjonctivite granuleuse à forme chronique. Par la culture pure de fragments d'une cornée malade, on obtient un mycélium.

12°. *Trichophyton ovoïdes*, Behrend, 1890.—Il cause la piedre ou trichomycose nodulaire et vit sur les cheveux ; en culture pure, il se développe en un mycélium.

13°. *Monilia sputicola*, Galippe, 1885.—Il a été trouvé dans la salive humaine ; il y passe inaperçu et y végète mal, se reproduisant sans doute uniquement par bourgeonnement des spores. Cultivé, il donne un riche mycélium dont les hyphes portent à leur extrémité des chapelets de spores.

Conclusions.

Les brillants travaux de Bazin et de ses élèves (école de Paris), puis d'Hebra et de ses élèves (école de Vienne) avaient fini par apporter

assez de lumière dans l'étude difficile des dermatomycoses, pour qu'on pût croire définitivement acquise cette notion, que les champignons de la peau sont essentiellement au nombre de quatre, causant chacun une maladie spéciale :

1°. Le *Microsporon furfur*, causant le pityriasis versicolor ;

2°. Le *Microsporon Audouini*, causant la pelade ;

3°. L'*Achorion Schoenleini*, causant la teigne faveuse ;

4°. Le *Trichophyton tonsurans*, causant les diverses formes de trichophytie et spécialement la teigne tondante.

Les recherches récemment faites en différents pays sont venues anéantir cette croyance. Non seulement le nombre des microphytes cuticoles a augmenté dans une large mesure, mais encore on a acquis la conviction que, sous les noms de favus et de trichophytie, on avait confondu plusieurs affections bien distinctes, caractérisées chacune par un organisme parasitaire spécifique.

Ces recherches sont encore trop récentes, elles sont d'ailleurs encore trop inachevées pour qu'on puisse prévoir où l'on s'arrêtera dans cette voie nouvelle et, par conséquent, pour qu'on puisse dès maintenant en tirer des conclusions définitives. Il est acquis néanmoins que des animaux d'espèce très variée (poule, bœuf, cheval, lapin, rat, souris, chien, chat) peuvent transmettre à l'homme, soit par une fréquentation assidue, soit par un contact passager, des maladies parasitaires capables de se développer chez celui-ci, d'y devenir plus ou moins tenaces et d'y provoquer des accidents plus ou moins graves.

Ces notions, basées tout à la fois sur l'observation clinique, sur la culture des microphytes en dehors de l'organisme et sur la reproduction expérimentale de la maladie, par inoculation soit de ces cultures, soit simplement de fragments épidermiques renfermant le parasite, rendent légitimes les conclusions suivantes :—

1°. Un certain nombre de dermatoses, causées par des champignons, sont transmises à l'homme par les animaux avec lesquels celui-ci peut se trouver en contact.

2°. Par la fréquentation journalière des animaux domestiques, l'homme est donc exposé à contracter certaines maladies cutanées.

3°. Tout animal dont la peau présente un aspect anormal (desquamation, croûtes, etc.) ou dont les poils semblent tomber ou se briser spontanément, doit être isolé, soumis à une stricte observation et traité par les procédés parasiticides.

4°. La litière ayant servi à cet animal sera détruite par le feu. Les stalles d'écurie, chenils, harnais, couvertures seront désinfectés. Les tapis, couvertures de lit, etc., sur lesquels les chats et les chiens se couchent volontiers, seront passés à l'étuve.

5°. L'homme peut lui-même communiquer certaines dermatomycoses aux animaux domestiques.

6°. Tant que le traitement médical n'aura pas arrêté le mal ou du moins ne l'aura pas suffisamment atténué pour que, de l'avis du médecin, toute chance de contamination ait disparu, on devra s'abstenir de tout contact avec les animaux, ceux-ci pouvant s'infester à leur tour et devenir ainsi, secondairement, les propagateurs de la maladie.

7°. Les différents gouvernements devront prohiber, par un règlement spécial, l'introduction sur leur territoire de tout animal de provenance étrangère, chez lequel on aura notoirement reconnu l'existence d'une dermatose parasitaire et transmissible, non seulement de l'animal à l'homme, mais aussi de l'animal à l'animal.

DISCUSSION.

Professor Brown, C.B., said: that after the very elaborate papers which had been already read, and particularly the last paper, which dealt so very minutely with some of the microphytes—indeed with a large number of species which were hardly known among scientists in this country—it would not be necessary for him to go into great detail. What he proposed to do was to discuss the subject generally, and endeavour to illustrate some of the remarks of previous speakers by the aid of microscopic lantern slides projected on the screen. It had always been perfectly well recognised that there were certain organisms which maintained an existence in other organisms. This condition was known under the general name of symbiosis, which simply meant association. The condition of parasitism was capable of being looked at from more than one point of view, and it was quite certain that parasitism included very various degrees of injury. He might remark that in numerous cases where outbreaks of disease amongst lambs during the past season had been ascribed to parasites, they had been able to show by the stern logic of facts that the parasites had nothing to do with the disturbance. They had found the same parasites in other lambs which were absolutely free from disease of any kind, and in the most flourishing condition. In other cases, however, parasites did inflict an enormous amount of mischief, and this was true, not only of the animal parasites, but also of those numerous microphytes which were derived from the vegetable kingdom, and which found a habitat generally on the surface of the higher animals, though occasionally in the internal organs.

Professor Brown then proceeded to describe in detail the characteristics of a variety of parasites by lantern demonstrations. Amongst the parasites thus described, were the following:—

Cestodes:
- Large cysts, *Tenuicollis.*
- Small cysts, *Echinococcus.*
- Tape worms, *Tœnia lata* (sheep),
- ,, ,, *Tœnia serrata* (dog).

Trematodes: Fluke, egg, embryro, sporocysts, cercaria.

Nematodes: Strongles, lung of sheep.

Acanthocephala: Thorn-headed worms.

In concluding, he observed that if he attempted to explain in detail the many ways in which animal parasites could be conveyed, he should only be quoting the words of Dr. Cobbold, who was certainly the greatest helminthologist of his day, at least in this country, and who had laid the basis of the work on which Englishmen had subsequently proceeded. He would only add that whilst parasites were naturally looked upon with a sentiment of disgust, and were charged with being the cause of every conceivable form of mischief, there was a point of view from which he apprehended they might be considered as suffering from injustice in the

struggle for existence. One could understand that a parasite like that on the screen might feel itself grossly illtreated by being expelled from the intestine in which it was placidly remaining, and it might allege on its own behalf, that it had as much right to infest the intestines of animals, as those animals had to infest or to live on others. There was no doubt, he admitted, that in many cases parasites did a considerable amount of mischief, and that in other instances they inflicted fatal injury; but at the same time the argument might be turned with terrible effect upon the human beings who complained of their ravages, if the question were fairly put to them : " Are you yourself free from that propensity to invade, " not only doing considerable mischief, but inflicting fatal injury, upon " your fellow worm ? "

Professor Perroncito (Turin) dit :—Je profite de l'occasion pour communiquer deux observations d'actinomycose de la peau qui peut-être doivent modifier les idées manifestées par mon très honoré ami et collègue Professeur Blanchard. L'une a été faite par moi avec notre très distingué M. Charles Reymond, Professeur d'Ophthalmologie à Turin; l'autre me l'a offert un Vétérinaire militaire avec M. le Dr. Perosino de Turin. Le premier cas se référe à une dame soignée par M. Reymond. Cette dame avait à une cuisse un nodule ulcéré qui résista à tout soin. M. Reymond a exporté une petite pièce de la tumeur dans laquelle nous avons trouvé les *cespugli* de l'*actinomycès* identiques à *l'actinomycès bovis*. Le second cas se référe à un cheval de l'artillerie, qui dans les manœuvres a été blessé à une cuisse avec une pierre. Le cheval a été mis dans une étable pour être soigné. Mais la plaie n'est jamais guérie complêtement; elle s'est au contraire mieux ouverte, et après une année le cheval a dû être tué par une actinomycose de la peau qui s'est transmise jusqu'à l'os.*

Ces deux faits démontrent comme l'actinomyces peut se transmettre pour la peau. Relativement aux parasites des animaux et plus spécialement les protozoaires, pour se transmettre entre les animaux, des animaux à l'homme et *vice versâ*, ils ont beaucoup de fois besoin de passer à l'état d'enkystément à l'état libre, précisément comme la trichine passe à l'état d'encapsulément dans les muscles des animaux. J'ai démontré dans l'année 1887 pour le *megastoma intestinalis*† et dans le 1888 pour les autres flagellés parasites de l'intestin de l'homme‡ que dans des conditions tout-à-fait spéciaux, comme j'ai expliqué dans mes communications annoncées, les flagelles surdits se revêtent d'une membrane kitineuse qui serve à transformer les cercomonas dans un état de vie durable, semblable à une spore d'un bactère. Ainsi les protozoaires peuvent résister à dessication et à d'autres vicissitudes atmosphériques pour rentrer dans le corps de l'homme ou des animaux et perpétuer l'espèce. Aussi dans le rat j'ai demontré spérimentalement que la *Lamblia intestinalis* de notre intestin administrée avec l'aliment, après six jours ont trouvé déjà dans les selles les megastoma encapsulés. Ce sont probablement les rats qui servent à propager l'espèce parasitaire dans l'homme. Un encapsulément analogue§; (je l'ai décrit dans le 1880), par les larves mûres de *l'Ankylostoma duodenalis*. Alors M. Leuckart, Grassi et autres ont objecté qu'on avait à faire plutôt

* Voir le livre par E. Perroncito : I Parassiti dell' uomo e degli animali utili. Milano, 1882.
† E. Perroncito: L'Enkystement du *megastoma intestinalis*. R. Academia di Medicina di Torino, Maggio, 1887.
‡ Sulla diffusione del *cercomonas intestinalis*. Turin, 1888.
§ *E. Perroncito* osservazioni elmintologoche relative all' anemia sooltassi epidemia vegli operai del Gottardo (R. Accademia dei Lincei—Seduta del 2 Maggio, 1880).

1968

RAI, J.N., AGARWAL, S.C. & TEWARI, J.P.

Cunninghamella brunnea spec. nov. - a possible product of ecological specialization.

J. gen. appl. Microbiol., Tokyo, 14 (4):443-446.

Microfilm no. 15610

J. Gen. Appl. Microbiol., 14, 443–446 (1968)

CUNNINGHAMELLA BRUNNEA SPEC. NOV.—A POSSIBLE PRODUCT OF ECOLOGICAL SPECIALIZATION

J.N. RAI, S.C. AGARWAL AND J.P. TEWARI

Botany Department, University of Lucknow, Lucknow, India

(Received August 28, 1968)

The name *Cunninghamella brunnea* spec. nov. has been given to a new phaeosporous merosporangiferous Mucorales isolated from '*Usar*' soils (pH 8.0–9.0), Lucknow, India. The new species is characterized by the presence of large greyish-brown spores and the absence of vesicles and oil-globules in the hyphae. This species is considered to have evolved as an ecological specialization in response to high temperature and intense solar radiation conditions prevailing in these soils.

Of the 12 species of Mucorales isolated during the course of detailed microbial analysis of '*Usar*' (alkaline) soils of Uttar Pradesh, 3 belonged to genus *Cunninghamella*, 2 each to *Absidia*, *Mucor* and *Rhizopus* and one each to *Circinella* and *Syncephalastrum*. The present paper deals with a new species of *Cunninghamella* named as *C. brunnea* spec. nov., the specific epithet being derived after the presence of greyish-brown spores.

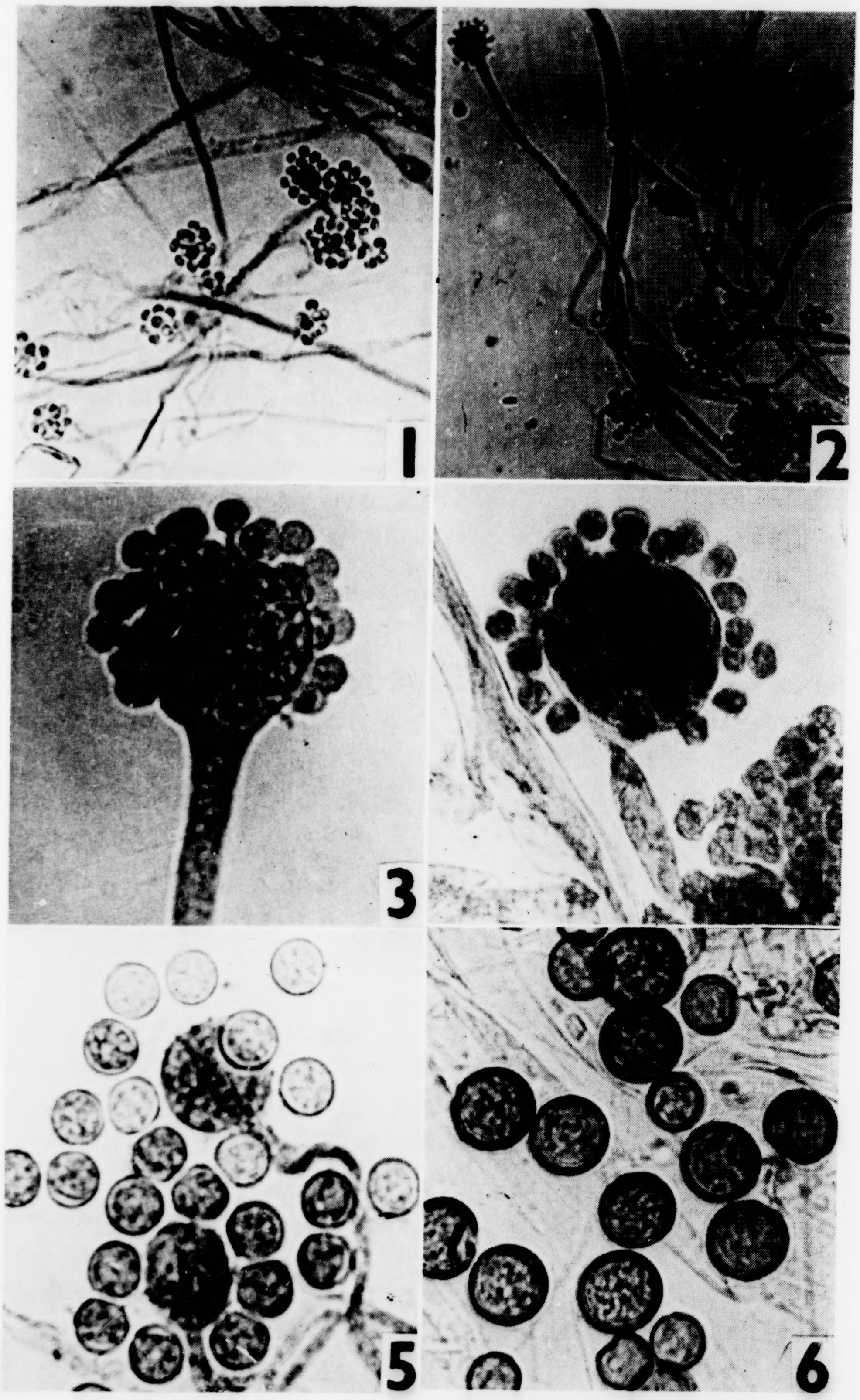
DESCRIPTION

Cunninghamella brunnea spec. nov.

Coloniae in agaro synthetico Mucoris rapide crescentes et attingentes diam. 6.7 cms. et tangetes operculum patinae petri dictae post dies 5 ad temp. 29±1°C, primo sordide albae, tum griseolae et tandem evadentes nigreolae in parte centrali ob productionem conidiorum, marginibus primo labis, tum griseis, et irregularibus, parte versa incolora; coloniae parte versa zonata et incolora. Hyphae hyalinae, 19.2 μ diam. Conidiophora recta, levia, hyalina, vulgo 12.8 μ crassa, producentia ramos laterales generatim verticillatos, qui ipsi terminantur vesiculis. Vesiculae hyalinae, parietibus tenuibus, subglobosae, vulgo 16.0–48.0 μ diam. Conidia vulgo sphaerica, hylina primo, ad maturitatem evadentia griseobrunnea, pulcherrime echinulate primo, tandem evadentia fere levia, magn. 9.6–24.0 μ diam., insidentia brevibus sterigmatibus.

Lectus mense julio 1967 ex Solo Usar (pH 8.0–9.0) ad Hindnagar, Lucknow. Culture typica posita in NRRL sub numero A-15, 753 in U.S.A. sectione agriculturae in U.S.A.

Colonies on Synthetic Mucor agar (SMA) growing rapidly, attaining a



Figs. 1-6. *Cunninghamella brunnea* spec. nov. on SMA.

1, 2: Fruiting structures, 1×94, 2×72. 3, 4: Young vesicles, 3×433, 4×519. 5: Vesicle with young hyaline conidia×519. 6: Mature greyish-brown conidia×693.

diameter of about 6.7 cm, and touching the lid of the Petri dish after 5 days' incubation at 29±1°C, at first dull white, then greyish and finally becoming blackish in the central regions due to the production of conidia, margins initially white, later turning greyish and irregular, reverse agar colourless, colony reverse zonate and colourless. Hyphae hyaline, up to 19.2 μ in width, devoid of oil-globules and vesicular swellings. Conidiophores straight, smooth, hyaline, generally up to 12.8 μ in width, producing lateral branches in verticils which in turn are also terminated by the vesicles. Vesicles hyaline, thin walled, subglobose, generally 16.0–48.0 μ in diameter. Conidia generally spherical, hyaline when young, turning greyish brown on maturity, very finely echinulate when young, later becoming almost smooth, measuring 9.6–24.0 μ in diameter, borne on short sterigmata. No zygospores produced.

Isolated in July, 1967 from '*Usar*' soil (pH 8.0–9.0) collected from Hindnagar, Lucknow. Type culture deposited as NRRL A-15, 753 in Northern Utilization Research and Development Division, U.S. Department of Agriculture, U.S.A.

DISCUSSION

The genus *Cunninghamella* comprises of a large number of species of which *C. homothallica* KOMINAMI and TUBAKI (*1*) and *C. phaeospora* BOEDIJN (*2*) have brown coloured spores. The latter species shows abundant oil-globules both in the hyphae and the prominent hyphal vesicles and 9.0–15.0 μ globose spores. The new species *C. brunnea* also falls in the phaeosporous section of the genus but differs in having 9.6–24.0 μ spores and the absence of vesicles and oil-globules in the hyphae. It also shows some resemblance to *C. elegans* Lendner (NAUMOV (*3*); Dr. C.W. Hesseltine and Dr. J.J. Ellis, *in litt.* Dec. 11, 1967) but differs in having larger, greyish-brown and only globose spores while the latter has both spherical and elliptical spores.

'*Usar*' (alkaline) soils from which this new species was isolated represent an extraordinary habitat in having high pH and osmotic concentrations. They also show very high soil surface temperature, low moisture content and high incidence of solar radiations specially during the summer. Isolation of this new species of *Cunninghamella* with dark-coloured spores from this habitat is remarkable and the third record in the family Cunninghamellaceae which is predominated by hyaline-spored fungi (*4*). This new species might have evolved as an ecological specialization in response to high temperatures and intense solar radiations to which these soils are subjected to. This is corroborated by DURRELL and SHIELDS' (*5*) findings on the soil fungi of Naveda Test Site and Death Valley, U.S.A., indicating that the dark-spored fungi predominate in these areas and that the melanin pigment serves a protective function. This role of pigments is also supported by the findings of STEVENS (*6*). DURRELL and FLEMING (*7*) described the new species *Thamnidium ctenidium* Durrell and Fleming from Death Valley showing

yellowish sporangiola with incrusted walls. This again seems to be an adaption to highly arid situations abounding in solar radiations where the soil surface temperature often reaches 190°F.

The authors are grateful to Dr. C.W. Hesseltine and Dr. J.J. Ellis for their valuable comments on the form described, to Rev. Fr. Prof. Dr. H. Santapau for latinizing the species diagnosis and to the U.S. PL 480 authorities for financing the work.

REFERENCES

1)* K. KOMINAMI, Y. KOBAYASI and K. TUBAKI, *Nagaoa*, **1**, 14 (1952).
2) K.B. BOEDIJN, *Sydowia*, **12**, 321 (1958).
3) N.A. NAUMOV, Clés des mucorinées (Mucorales). Paul Lechevalier, Paris, pp. 116.
4) C.W. HESSELTINE, *Mycologia*, **47**, 344 (1955).
5) L.W. DURRELL and L.M. SHIELDS, *Mycologia*, **52**, 636 (1960).
6)* F.L. STEVENS, *The Phill. Agric.*, **19**, 491 (1931).
7) L.W. DURRELL and M. FLEMING, *Mycologia*, **58**, 797 (1966).

* Originals not consulted.

SHORT COMMUNICATION

J. Gen. Appl. Microbiol., **14**, 447–450 (1968)

THE UTILIZATION OF METHANE, ETHANE AND PROPANE BY SOIL MICRO-ORGANISMS

P.G. BRISBANE AND J.N. LADD

C.S.I.R.O. Division of Soils, Adelaide, South Australia

(Received June 11, 1968)

The bacteria obtained from soils enriched with gaseous alkanes have been studied by various workers (*1–4*). The substrate specificity of these bacteria is of considerable interest as LEADBETTER and FOSTER (*5*) have shown that *Pseudomonas methanica* will not grow on ethane, propane and butane but will co-oxidise these alkanes whilst growing on methane. This paper describes the utilization of mixtures of gaseous alkanes by the micro-flora of a wide range of soils.

Duplicate top-soil samples were collected from seventeen sites at eleven locations near Adelaide. At three sites sub-soils were also sampled. Within ten days of collection, the soils (Table 1) were assayed for their ability to oxidise gaseous alkanes using the following method.

Each sample of soil was sieved and mixed. A sub-sample (20 g) was placed in a bottle (250 ml capacity), covered with 30 ml of mineral salt solution and sealed with a rubber bung fitted with a glass tube stoppered by a SUBASEAL* (*6, 7*). The bottles were evacuated and filled with a gas mixture containing 0.6% methane, 0.3% ethane and 0.2% propane in air and incubated as sets of shaken and still cultures at 25° for 69 days. After 21 and 45 days' incubation, all bottles were re-evacuated and refilled with the mixture of gaseous substrates. In addition, five soils were incubated as shaken cultures with individual alkanes at concentrations of 0.6% methane, or 0.3% ethane or 0.2% propane in air. Controls contained alkane(s) and mineral salt solution but no soil.

At least once each week a 250 μl sample of the atmosphere within each bottle was removed with a hypodermic syringe, via the Subaseal stopper, and injected into a F & M model 609 flame ionization gas chromatograph. The alkanes were separated at room temperature on a column containing 30% (w/w) silicone oil DC 200 supported on Chromasorb P. Alkane concentrations were estimated by measuring peak heights (*6, 7*).

* Subaseal stoppers obtained from W. Freeman & Co., Ltd., Barnsley, Yorks, U.K.

K. LAUGASTE

SEASONAL DYNAMICS OF THE RELATIVE WEIGHT, GLYCOGEN AND FAT CONTENT OF BREAM LIVER

Summary

In the course of 19 months, from April, 1966 to October, 1967, an analysis was carried out of 296 bream of an age of 5—17 years, and average length of 301 mm, caught in Lake Võrtsjärv. Of the lot, 88 were male, 117 female and 91 juvenile fish. They were analysed once a month at least, more frequently — during the period of spawning, and more rarely — in winter. The relative weight of the liver (further on — liver index) was calculated in per cent of the weight of the fish, without internal organs. The glycogen and fat content was evaluated visually, from histological preparations, according to a 5-mark scale. For evaluating the amount of glycogen, the material was fixed in Rossman's fixator, and embedded in paraffin, and 5 µm-thick slices were stained by the PAS reaction. For staining the fat, sudan black B, dissolved either in propylene-glycole or in 70 per cent spirit was applied after the preliminary fixation of the material in the Baker's fixator.

Both the liver index and the content of fat and glycogen are subject to considerable seasonal changes. The liver index fluctuated within the limits of 0.49—4.02 per cent, the average being 1.74 per cent. The largest liver index was in April: in females — 2.41 per

PARMASTO, E. 1969

Kortitsievye griby Sovetskogo Soyuza. VII.
[Corticiaceae of the Soviet Union.]

Eesti NSV Tead. Akad. Toim., Biol., 18 (4):387-389.
[1 table. Eston. summ.]

Microfilm no.

ulator of the above-mentioned changes, since it is in good correlation with all the indexes, the correlation with the fat content being positive (correlation coefficient +0.72), and the correlation with the glycogen content and liver index — negative (correlation coefficients —0.70 and —0.54, respectively). The changes in the liver of the sexually mature and immature fish proceed parallelly, as well.

Academy of Sciences of the Estonian SSR,
Institute of Zoology and Botany

Received
Dec. 4, 1968

EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. XVIII KÖIDE
BIOLOOGIA. 1969, nr. 4

ИЗВЕСТИЯ АКАДЕМИИ НАУК ЭСТОНСКОЙ ССР. ТОМ XVIII
БИОЛОГИЯ. 1969, № 4

Э. ПАРМАСТО

КОРТИЦИЕВЫЕ ГРИБЫ СОВЕТСКОГО СОЮЗА. VII
(*CORTICIACEAE U.R.S.S. VII*)

В седьмой части обзора кортициевых грибов СССР продолжен список грибов, издаваемых в эксикатах «Corticiaceae U.R.S.S.». Тираж эксикатов ограничен восемью экземплярами, которые высылаются в научные учреждения, указанные во второй части обзора (Изв. АН ЭССР, Сер. биол. **14** (3) : 317). Описания новых таксонов, представленные в эксикатах, опубликованы в книге автора «Конспект системы кортициевых грибов» (Тарту, 31/VII 1968 г.).

Как в предыдущие части, так и в настоящий выпуск эксикатов включены кортициоидные грибы не только семейства *Corticiaceae* s. str., но и близких семейств (*Stereaceae*, *Steccherinaceae*, *Echinodontiaceae*), а также «несовершенные» стадии кортициевых грибов.

In septima parte conspectus Corticiacearum U.R.S.S. index fungorum sequitur, qui in exsiccato „Corticiaceae U.R.S.S." eduntur. Exemplaria iterum VIII eius exsiccati mituntur ad instituta litterarum notata in parte altera huius operis (vide Eesti NSV Teaduste Akadeemia Toimetised, Bioloogiline Seeria **14** (3) : 317). Descriptiones taxorum novorum in hoc exsiccato expositorum in „Conspectu systematis Corticiacearum" auctoris (1968) editae sunt.

Ut in partibus prioribus, ita in hac quoque centuria exsiccati non tantum ad familiam *Corticiacearum* s. str. pertinentes, sed etiam alii fungi corticioidei ex familiis affinibus (*Stereaceae, Steccherinaceae, Echinodontiaceae*) una cum stadiis „imperfectis" eorum exponuntur.

INDEX

301. *Aegerita candida* Fr.
302. *Aegerita candida* Fr.
303. *Amphinema byssoides* (Fr.) John Erikss.
304. *Amphinema byssoides* (Fr.) John Erikss.
305. *Amphinema byssoides* (Fr.) John Erikss.
306. *Amylocorticium cebennense* (Bourd.) Pouz.
307. *Amylocorticium suaveolens* Parm. (i s o t y p u s!)
308. *Amylocorticium subincarnatum* (Peck) Pouz.
309. *Amylocorticium subincarnatum* (Peck) Pouz.
310. *Amylocorticium subincarnatum* (Peck) Pouz. f. *crassa* Parm. (i s o t y p u s!)
311. *Amylostereum areolatum* (Fr.) Boid.
312. *Amylostereum Chailletii* (Fr.) Boid.
313. *Amylostereum Chailletii* (Fr.) Boid.
314. *Amylostereum Chailletii* (Fr.) Boid.
315. *Amylostereum laevigatum* (Fr.) Boid.

316. *Amylostereum laevigatum* (Fr.) Boid.
317. *Auriculariopsis ampla* (Lév.) Maire
318. *Boreostereum radiata* (Peck) Parm.
319. *Boreostereum radiata* (Peck) Parm.
320. *Chaetocorticium luna* (Rom.) Parm.
321. *Chaetocorticium luna* (Rom.) Parm.
322. *Columnocystis abietina* (Fr.) Pouz.
323. *Columnocystis abietina* (Fr.) Pouz.
324. *Columnocystis ambigua* (Peck) Pouz.
325. *Cystidiophorus merulioideus* Bond. et Ljub.
326. *Cystidiophorus merulioideus* Bond. et Ljub.
327. *Cystostereum Murraii* (Berk. et Curt.) Pouz.
328. *Cystostereum Murraii* (Berk. et Curt.) Pouz.
329. *Cystostereum Murraii* (Berk. et Curt.) Pouz.
330. *Cystostereum pini-canadense* (Schw.) Parm.
331. *Cystostereum pini-canadense* (Schw.) Parm.
332. *Dacryobolus Karstenii* (Bres.) Oberw.
333. *Dacryobolus Karstenii* (Bres.) Oberw.
334. *Epithele typhae* (Fr.) Pat.
335. *Fibricium Greschikii* (Bres.) John Erikss.
336. *Fibricium Greschikii* (Bres.) John Erikss.
337. *Fibricium lapponicum* John Erikss.
338. *Fibrodontia gossypina* Parm. (p a r a t y p u s!)
339. *Hyphoderma argillaceum* (Bres.) Donk
340. *Hyphoderma mutatum* (Peck) Donk
341. *Hyphoderma mutatum* (Peck) Donk
342. *Hyphoderma polonense* (Bres.) Donk
343. *Hyphoderma setigerum* (Fr.) Donk
344. *Hyphoderma setigerum* (Fr.) Donk
345. *Hyphoderma setigerum* (Fr.) Donk
346. *Hyphoderma tenue* (Pat.) Donk
347. *Hyphoderma tenue* (Pat.) Donk
348. *Hyphoderma tenue* (Pat.) Donk
349. *Hyphodontia Nikolajevae* Parm. (i s o t y p u s!)
350. *Hyphodontia pallidula* (Bres.) John Erikss. var. *alutarioides* Parm. (p a r a t y p u s!)
351. *Laurilia sulcata* (Burt) Pouz.
352. *Laurilia sulcata* (Burt) Pouz.
353. *Lopharia mirabilis* (Berk. et Br.) Pat.
354. *Lopharia spadicea* (Fr.) Boid.
355. *Lopharia spadicea* (Fr.) Boid.
356. *Metulodontia farinosa* (Bres.) Parm.
357. *Metulodontia junquillea* (Quél.) Parm.
358. *Metulodontia nivea* (Karst.) Parm.
359. *Metulodontia nivea* (Karst.) Parm.
360. *Metulodontia pruni* (Lasch) Parm.
361. *Peniophora aurantiaca* (Bres.) Höhn. et Litsch.
362. *Peniophora aurantiaca* (Bres.) Höhn. et Litsch.
363. *Peniophora Erikssonii* Boid.
364. *Peniophora Erikssonii* Boid.
365. *Peniophora incarnata* (Fr.) Karst.
366. *Peniophora incarnata* (Fr.) Karst.
367. *Peniophora junipericola* John Erikss.
368. *Peniophora lilacea* Bourd. et Galz.
369. *Peniophora limitata* (Fr.) Cke.
370. *Peniophora limitata* (Fr.) Cke.
371. *Peniophora Lycii* (Pers.) Höhn. et Litsch.
372. *Peniophora Lycii* (Pers.) Höhn. et Litsch.
373. *Peniophora piceae* (Pers.) John Erikss.
374. *Peniophora piceae* (Pers.) John Erikss.
375. *Peniophora pithya* (Pers.) John Erikss.
376. *Peniophora pithya* (Pers.) John Erikss.
377. *Peniophora pini* (Fr.) Boid.
378. *Peniophora pini* (Fr.) Boid.
379. *Peniophora proxima* Bres.
380. *Peniophora quercina* (Fr.) Cke.
381. *Peniophora rufa* (Fr.) Boid.
382. *Peniophora rufa* (Fr.) Boid.
383. *Peniophora septentrionalis* Laur.
384. *Peniophora versiformis* (Berk. et Curt.) Bourd. et Galz.
385. *Peniophora versiformis* (Berk. et Curt.) Bourd. et Galz.
386. *Peniophora violaceo-livida* (Sommerf.) Massee
387. *Pulcherricium caeruleum* (Fr.) Parm.
388. *Pulcherricium caeruleum* (Fr.) Parm.
389. *Resinicium bicolor* (Fr.) Parm.
390. *Resinicium bicolor* (Fr.) Parm.
391. *Resinicium bicolor* (Fr.) Parm.
392. *Sistotrema Brinkmannii* (Bres.) John Erikss.
393. *Sistotremastrum suecicum* Litsch.
394. *Stereum gausapatum* (Fr.) Fr.
395. *Stereum gausapatum* (Fr.) Fr.
396. *Stereum insignitum* Quél.
397. *Stereum rugosum* (Fr.) Fr.
398. *Stereum rugosum* (Fr.) Fr.
399. *Subulicystidium longisporum* (Pat.) Parm.
400. *Subulicystidium longisporum* (Pat.) Parm.

Институт зоологии и ботаники
Академии наук Эстонской ССР

Поступила в редакцию
12/XI 1968

E. PARMASTO

SUGUKOND *CORTICIACEAE* NSV LIIDUS. VII

Resümee

Ülevaate seitsmendas osas jätkub eksikaatkogus «*Corticiaceae* U.R.S.S.» sisalduvate seente nimestik. Eksikaatkogu saadetakse kaheksale tähtsamale seente herbaariumile (vt. Eesti NSV Teaduste Akadeemia Toimetised, Bioloogiline Seeria **14** (3) : 317).

Eksikaatkogus esitatakse mitmed teadusele uued taksoonid, mille kirjeldused on avaldatud autori töös «Conspectus systematis *Corticiacearum*» (Tartu, 1968).

Eesti NSV Teaduste Akadeemia
Zooloogia ja Botaanika Instituut

Saabus toimetusse
12. XI 1968

K. LAUGASTE

SEASONAL DYNAMICS OF THE RELATIVE WEIGHT, GLYCOGEN AND FAT CONTENT OF BREAM LIVER

Summary

In the course of 19 months, from April, 1966 to October, 1967, an analysis was carried out of 296 bream of an age of 5—17 years, and average length of 301 mm, caught in Lake Võrtsjärv. Of the lot, 88 were male, 117 female and 91 juvenile fish. They were analysed once a month at least, more frequently — during the period of spawning, and more rarely — in winter. The relative weight of the liver (further on — liver index) was calculated in per cent of the weight of the fish, without internal organs. The glycogen and fat content was evaluated visually, from histological preparations, according to a 5-mark scale. For evaluating the amount of glycogen, the material was fixed in Rossman's fixator, and embedded in paraffin, and 5 µm-thick slices were stained by the PAS reaction. For staining the fat, sudan black B, dissolved either in propylene-glycole or in 70 per cent spirit was applied after the preliminary fixation of the material in the Baker's fixator.

Both the liver index and the content of fat and glycogen are subject to considerable seasonal changes. The liver index fluctuated within the limits of 0.49—4.02 per cent, the average being 1.74 per cent. The largest liver index was in April: in females — 2.41 per

1969

PARMASTO, E.

Kortitsievȳe gribȳ Sovetskogo Soyuza. VII.
/Corticiaceae of the Soviet Union./

Eesti NSV Tead. Akad. Toim., Biol., 18 (4):387-389.
/1 table. Eston. summ./

Microfilm no. 15612

ulator of the above-mentioned changes, since it is in good correlation with all the indexes, the correlation with the fat content being positive (correlation coefficient +0.72), and the correlation with the glycogen content and liver index — negative (correlation coefficients —0.70 and —0.54, respectively). The changes in the liver of the sexually mature and immature fish proceed parallelly, as well.

Academy of Sciences of the Estonian SSR,
Institute of Zoology and Botany

Received
Dec. 4, 1968

EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. XVIII KÖIDE
BIOLOOGIA. 1969, nr. 4

ИЗВЕСТИЯ АКАДЕМИИ НАУК ЭСТОНСКОЙ ССР. ТОМ XVIII
БИОЛОГИЯ. 1969, № 4

Э. ПАРМАСТО

КОРТИЦИЕВЫЕ ГРИБЫ СОВЕТСКОГО СОЮЗА. VII
(CORTICIACEAE U.R.S.S. VII)

В седьмой части обзора кортициевых грибов СССР продолжен список грибов, издаваемых в эксикатах «Corticiaceae U.R.S.S.». Тираж эксикатов ограничен восемью экземплярами, которые высылаются в научные учреждения, указанные во второй части обзора (Изв. АН ЭССР, Сер. биол. **14** (3) : 317). Описания новых таксонов, представленные в эксикатах, опубликованы в книге автора «Конспект системы кортициевых грибов» (Тарту, 31/VII 1968 г.).

Как в предыдущие части, так и в настоящий выпуск эксикатов включены кортициоидные грибы не только семейства *Corticiaceae* s. str., но и близких семейств (*Stereaceae*, *Steccherinaceae*, *Echinodontiaceae*), а также «несовершенные» стадии кортициевых грибов.

In septima parte conspectus Corticiacearum U.R.S.S. index fungorum sequitur, qui in exsiccato „Corticiaceae U.R.S.S." eduntur. Exemplaria iterum VIII eius exsiccati mituntur ad instituta litterarum notata in parte altera huius operis (vide Eesti NSV Teaduste Akadeemia Toimetised, Bioloogiline Seeria **14** (3) : 317). Descriptiones taxorum novorum in hoc exsiccato expositorum in „Conspectu systematis Corticiacearum" auctoris (1968) editae sunt.

Ut in partibus prioribus, ita in hac quoque centuria exsiccati non tantum ad familiam *Corticiacearum* s. str. pertinentes, sed etiam alii fungi corticioidei ex familiis affinibus (*Stereaceae, Steccherinaceae, Echinodontiaceae*) una cum stadiis „imperfectis" eorum exponuntur.

INDEX

301. *Aegerita candida* Fr.
302. *Aegerita candida* Fr.
303. *Amphinema byssoides* (Fr.) John Erikss.
304. *Amphinema byssoides* (Fr.) John Erikss.
305. *Amphinema byssoides* (Fr.) John Erikss.
306. *Amylocorticium cebennense* (Bourd.) Pouz.
307. *Amylocorticium suaveolens* Parm. (i s o t y p u s!)
308. *Amylocorticium subincarnatum* (Peck) Pouz.
309. *Amylocorticium subincarnatum* (Peck) Pouz.
310. *Amylocorticium subincarnatum* (Peck) Pouz. f. *crassa* Parm. (i s o t y p u s!)
311. *Amylostereum areolatum* (Fr.) Boid.
312. *Amylostereum Chailletii* (Fr.) Boid.
313. *Amylostereum Chailletii* (Fr.) Boid.
314. *Amylostereum Chailletii* (Fr.) Boid.
315. *Amylostereum laevigatum* (Fr.) Boid.

316. *Amylostereum laevigatum* (Fr.) Boid.
317. *Auriculariopsis ampla* (Lév.) Maire
318. *Boreostereum radiata* (Peck) Parm.
319. *Boreostereum radiata* (Peck) Parm.
320. *Chaetocorticium luna* (Rom.) Parm.
321. *Chaetocorticium luna* (Rom.) Parm.
322. *Columnocystis abietina* (Fr.) Pouz.
323. *Columnocystis abietina* (Fr.) Pouz.
324. *Columnocystis ambigua* (Peck) Pouz.
325. *Cystidiophorus merulioideus* Bond. et Ljub.
326. *Cystidiophorus merulioideus* Bond. et Ljub.
327. *Cystostereum Murraii* (Berk. et Curt.) Pouz.
328. *Cystostereum Murraii* (Berk. et Curt.) Pouz.
329. *Cystostereum Murraii* (Berk. et Curt.) Pouz.
330. *Cystostereum pini-canadense* (Schw.) Parm.
331. *Cystostereum pini-canadense* (Schw.) Parm.
332. *Dacryobolus Karstenii* (Bres.) Oberw.
333. *Dacryobolus Karstenii* (Bres.) Oberw.
334. *Epithele typhae* (Fr.) Pat.
335. *Fibricium Greschikii* (Bres.) John Erikss.
336. *Fibricium Greschikii* (Bres.) John Erikss.
337. *Fibricium lapponicum* John Erikss.
338. *Fibrodontia gossypina* Parm. (paratypus!)
339. *Hyphoderma argillaceum* (Bres.) Donk
340. *Hyphoderma mutatum* (Peck) Donk
341. *Hyphoderma mutatum* (Peck) Donk
342. *Hyphoderma polonense* (Bres.) Donk
343. *Hyphoderma setigerum* (Fr.) Donk
344. *Hyphoderma setigerum* (Fr.) Donk
345. *Hyphoderma setigerum* (Fr.) Donk
346. *Hyphoderma tenue* (Pat.) Donk
347. *Hyphoderma tenue* (Pat.) Donk
348. *Hyphoderma tenue* (Pat.) Donk
349. *Hyphodontia Nikolajevae* Parm. (isotypus!)
350. *Hyphodontia pallidula* (Bres.) John Erikss. var. *alutarioides* Parm. (paratypus!)
351. *Laurilia sulcata* (Burt) Pouz.
352. *Laurilia sulcata* (Burt) Pouz.
353. *Lopharia mirabilis* (Berk. et Br.) Pat.
354. *Lopharia spadicea* (Fr.) Boid.
355. *Lopharia spadicea* (Fr.) Boid.
356. *Metulodontia farinosa* (Bres.) Parm.
357. *Metulodontia junquillea* (Quél.) Parm.
358. *Metulodontia nivea* (Karst.) Parm.
359. *Metulodontia nivea* (Karst.) Parm.
360. *Metulodontia pruni* (Lasch) Parm.
361. *Peniophora aurantiaca* (Bres.) Höhn. et Litsch.
362. *Peniophora aurantiaca* (Bres.) Höhn. et Litsch.
363. *Peniophora Erikssonii* Boid.
364. *Peniophora Erikssonii* Boid.
365. *Peniophora incarnata* (Fr.) Karst.
366. *Peniophora incarnata* (Fr.) Karst.
367. *Peniophora junipericola* John Erikss.
368. *Peniophora lilacea* Bourd. et Galz.
369. *Peniophora limitata* (Fr.) Cke.
370. *Peniophora limitata* (Fr.) Cke.
371. *Peniophora Lycii* (Pers.) Höhn. et Litsch.
372. *Peniophora Lycii* (Pers.) Höhn. et Litsch.
373. *Peniophora piceae* (Pers.) John Erikss.
374. *Peniophora piceae* (Pers.) John Erikss.
375. *Peniophora pithya* (Pers.) John Erikss.
376. *Peniophora pithya* (Pers.) John Erikss.
377. *Peniophora pini* (Fr.) Boid.
378. *Peniophora pini* (Fr.) Boid.
379. *Peniophora proxima* Bres.
380. *Peniophora quercina* (Fr.) Cke.
381. *Peniophora rufa* (Fr.) Boid.
382. *Peniophora rufa* (Fr.) Boid.
383. *Peniophora septentrionalis* Laur.
384. *Peniophora versiformis* (Berk. et Curt.) Bourd. et Galz.
385. *Peniophora versiformis* (Berk. et Curt.) Bourd. et Galz.
386. *Peniophora violaceo-livida* (Sommerf.) Massee
387. *Pulcherricium caeruleum* (Fr.) Parm.
388. *Pulcherricium caeruleum* (Fr.) Parm.
389. *Resinicium bicolor* (Fr.) Parm.
390. *Resinicium bicolor* (Fr.) Parm.
391. *Resinicium bicolor* (Fr.) Parm.
392. *Sistotrema Brinkmannii* (Bres.) John Erikss.
393. *Sistotremastrum suecicum* Litsch.
394. *Stereum gausapatum* (Fr.) Fr.
395. *Stereum gausapatum* (Fr.) Fr.
396. *Stereum insignitum* Quél.
397. *Stereum rugosum* (Fr.) Fr.
398. *Stereum rugosum* (Fr.) Fr.
399. *Subulicystidium longisporum* (Pat.) Parm.
400. *Subulicystidium longisporum* (Pat.) Parm.

Институт зоологии и ботаники
Академии наук Эстонской ССР

Поступила в редакцию
12/XI 1968

E. PARMASTO

SUGUKOND *CORTICIACEAE* NSV LIIDUS. VII

Resümee

Ülevaate seitsmendas osas jätkub eksikaatkogus «*Corticiaceae* U.R.S.S.» sisalduvate seente nimestik. Eksikaatkogu saadetakse kaheksale tähtsamale seente herbaariumile (vt. Eesti NSV Teaduste Akadeemia Toimetised, Bioloogiline Seeria **14** (3) : 317).

Eksikaatkogus esitatakse mitmed teadusele uued taksoonid, mille kirjeldused on avaldatud autori töös «Conspectus systematis *Corticiacearum*» (Tartu, 1968).

Eesti NSV Teaduste Akadeemia
Zooloogia ja Botaanika Instituut

Saabus toimetusse
12. XI 1968

ствуют аналогично бифункциональным. Ингибирование синтеза ДНК легко снимается при удалении препарата отмывкой клеток. Молекулярный механизм ингибирующего действия диазокетонов остается в целом неясным. По-видимому, ингибирование синтеза макромолекул может быть причиной мутагенного и противоопухолевого действия диазокетонов.

Филиал Института химической физики
Академии наук СССР
Черноголовка Московской обл.

Поступило
9 II 1969

ЦИТИРОВАННАЯ ЛИТЕРАТУРА

[1] И. А. Рапопорт, ДАН, 130, 1134 (1960). [2] Л. Л. Гуманов, С. Д. Кононова, Н. П. Норенко, Супермутагены, «Наука», 1966, стр. 49. [3] Н. М. Эмануэль, Н. П. Коновалова и др., ДАН, 183, 724 (1968). [4] F. Arndt, J. Amende, Ber., 61, 1122 (1928).

1969

RAGOZINA, I.I., SHNEIDER, YU. I. & LIPSITS, D.V.

Pektoliticheskie fermenty v kul'ture Pectobacterium phytophthorum i v porazhennykh eyu tkanyakh Kartofelya.

[Pectolytic ferments in Erwinia atroseptica culture and Potato tissue infected by it.]

Dokl. Akad. Nauk SSSR, 188 (4):937-939. [4 graphs, 1 table. Res. Inst. Potato Farming, Kraskovo, Moscow Dist.]

Microfilm no. 15613

Доклады Академии наук СССР
1969. Том 188, № 4

УДК 581.2 ***ФИТОПАТОЛОГИЯ***

И. И. РАГОЗИНА, Ю. И. ШНЕЙДЕР, Д. В. ЛИПСИЦ

ПЕКТОЛИТИЧЕСКИЕ ФЕРМЕНТЫ В КУЛЬТУРЕ PECTOBACTERIUM PHYTOPHTHORUM И В ПОРАЖЕННЫХ ЕЮ ТКАНЯХ КАРТОФЕЛЯ

(Представлено академиком А. И. Опариным 8 IV 1969)

Ферменты пектинового комплекса (пектинметилэстераза — ПМЭ, полигалактуроназа — ПГ, пектинтрансэлиминаза — ПТЭ и др.) широко распространены в мире микроорганизмов ([1]). С их образованием часто связывают патогенность многих из возбудителей заболеваний ([2–8]). Однако вопрос о роли пектолитических ферментов (п.ф.) в патогенезе далеко еще не выяснен. В ряде случаев какой-либо корреляции между активностью п.ф. и патогенностью изолятов не удалось обнаружить ([9–11]). Известно также, что многие непатогенные формы микроорганизмов также продуцируют п.ф. и мацерируют ткани in vitro ([12]). Результаты, полученные при культивировании патогенов на искусственных средах, содержащих пектиновые вещества, отнюдь не всегда отражают их ферментативную активность в пораженных тканях растения-хозяина. Для решения этого вопроса необходимы систематические определения активности п.ф. и других ферментативных систем в зараженных тканях — в динамике и в самые ранние сроки после заражения. Такое исследование предпринято в связи с изучением устойчивости картофеля к возбудителю черной ножки P. phytophthorum.

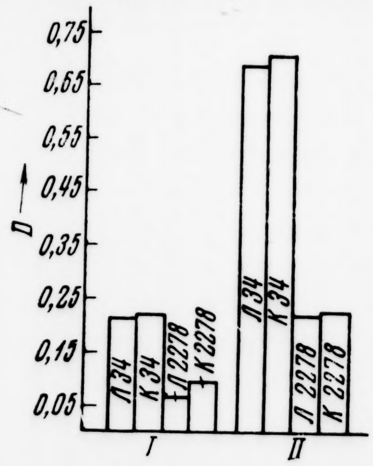


Рис. 1

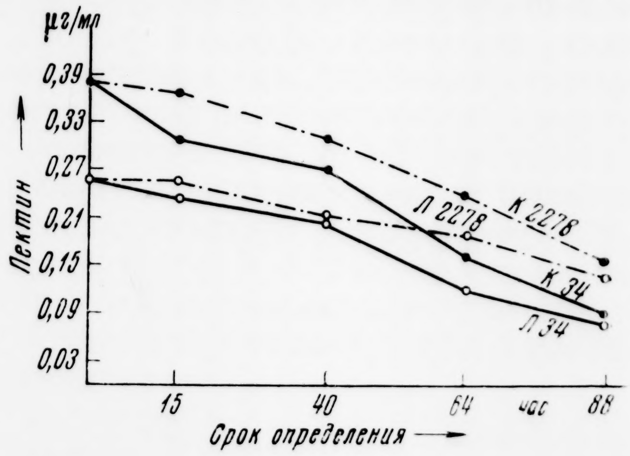


Рис. 2

Рис. 1. Активность ПТЭ в культуральных фильтратах, в зависимости от содержания в среде пектина (20-часовая культура). *Л* — на отваре из клубней сорта Любимец, *К* — на отваре из клубней сорта Камераз. *I* — контроль, *II* — 0,5% пектина

Рис. 2. Изменение количества пектина в культуральных фильтрах

Первые результаты ([13]) показали, что степень патогенности различных изолятов P. phytophthorum коррелирует с активностью продуцируемых ею протеиназ; вместе с тем активность этих экзопротеиназ в зараженных тканях клубней картофеля находится в обратной зависимости от степени устойчивости сорта к черной ножке. В настоящем сообщении приводятся данные об активности п.ф. как в культуральных фильтратах P. phytophthorum (вирулентного штамма 34 и слабовирулентного 2278), так и в зараженных тканях двух сортов картофеля: Камераз, обладающего повышенной устойчивостью, и Любимец — высоковосприимчивого.

Бактерии культивировали на отварах из клубней сортов Камераз и Любимец, к которым добавляли 0,5% пектина (фирмы Лоусон, Англия); на 100 мл среды вводили по 2 млрд бактериальных клеток и инкубировали

при 30°. Отделенные в фазу бутонизации стебли заражали, опуская их концы (0,5 см) в суспензию бактерий (100 млн/мл), налитую в колбу. Через 1; 24; 48 и 72 часа после заражения от нижней части стеблей отделяли для анализов по 3 трехсантиметровых отрезка. Контролем служили стебли, опущенные в воду. Цилиндры из сердцевинной части клубня ($d = 8$ *мм*, $l = 40$ мм) заражали в пробирках с плоским дном, опуская их верхушечной частью в суспензию бактерий (0,2 мл, 50 млн/мл) и инкубируя затем в термостате при 15°. Через 1; 8; 24; 32 и 48 час. после заражения от цилиндра отделяли снизу 4 зоны, по 0,5 см каждая.

Активность ПМЭ определяли по степени подкисления среды ([14]). Для получения ферментного раствора растирали на холоду в присутствии метабисульфита калия (0,15%) 1 г ткани с 5 мл 0,25 *N* NaCl, центрифугировали и диализовали экстракт в течение 16—18 час. против воды. Активность ПГ

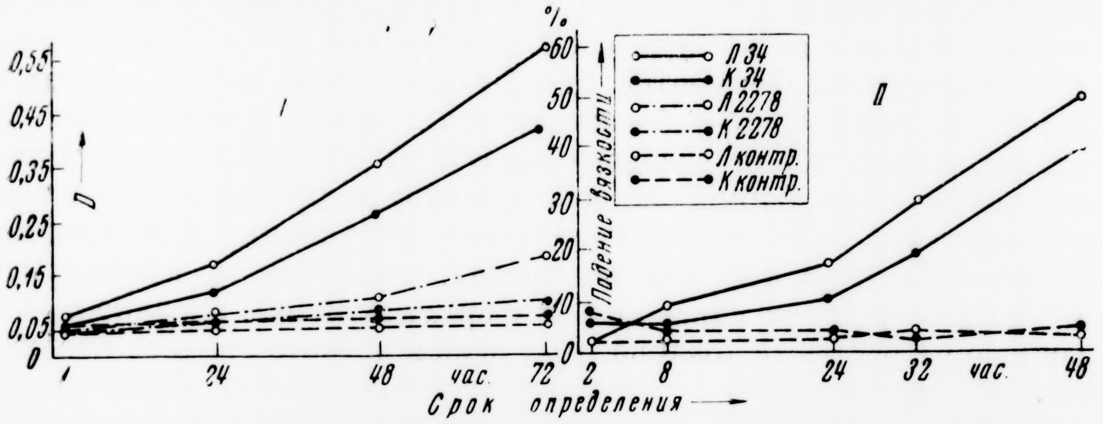


Рис. 3. Активность ПТЭ в тканях зараженных стеблей (*I*) и клубней (*II*) картофеля. Обозначения те же, что на рис. 1

определяли вискозиметрически ([14]). Фермент экстрагировали в тех же условиях ацетатным буфером рН 5,2. Активность ПТЭ определяли спектрофотометрически, при помощи тиобарбитуровой кислоты, которая с продуктами распада пектина дает окрашенные соединения с максимумом поглощения при 550 mμ ([15]), а также по степени снижения вязкости ([20]). Ферментный раствор получали растирая на холоду 1 г ткани с 5 мл 0,1 *M* трис-HCl-буфера рН 8,6. Экстракт диализовали в течение ночи против воды.

Активность ферментов в культуральных фильтратах определяли через 0; 14; 40 и 72 часа после посева. Для отделения бактериальных клеток применяли центрифугирование при 10 тыс. об/мин в течение 15 мин. Фильтраты диализовали в течение ночи против воды. Субстратом при всех определениях служил 1% раствор пектина. Количество пектина в культуральных фильтратах определяли карбазоловым методом ([16]).

Рис. 4. Активность ПТЭ в тканях различных зон клубня картофеля, через 48 час. после заражения. Обозначения те же, что на рис. 1

Как оказалось, в культуральных фильтратах P. phytophthorum можно было обнаружить только активность ПТЭ, которая отчетливо коррелировала с вирулентностью штамма, а в присутствии пектина возрастала более чем в 3 раза (рис. 1). Благодаря этому наблюдалась значительная разница в изменении содержания пектина в среде при культивировании двух штаммов, различающихся по вирулентности (рис. 2).

В здоровых и зараженных тканях стеблей и клубней совершенно отсутствовала активность ПГ. Активность ПМЭ была значительно выше в тканях стеблей, чем в тканях клубней; в ответ на заражение она менялась очень мало. Наибольший интерес представляют данные об активности ПТЭ в зараженных тканях в разные сроки

после заражения (рис. 3). Этот фермент стал известен совсем недавно ([17]). Он осуществляет так называемое трансэлиминативное (не гидролитическое) расщепление пектина по глюкозидной связи α-1,4, с образованием ненасыщенных продуктов. В последние годы ПТЭ была обнаружена у многих сапрофитов, факультативных паразитов и симбионтов; ее активность в большой степени зависит от pH среды и концентрации ионов Ca ([18], [19]).

В первые сроки определений, когда видимые симптомы заболевания еще отсутствуют, активность ПТЭ в зараженных тканях стеблей и клубней очень невелика. Лишь через 24 часа она начинает резко повышаться, причем в большей степени в тканях восприимчивого сорта. Это повышение активности совпадает по времени с появлением видимых признаков мацерации ткани, что можно иллюстрировать данными, характеризующими активность ПТЭ в исследуемых зонах клубня, через 48 час. после заражения (рис. 4). Важно отметить, что после заражения тканей стеблей и клубней слабовирулентным штаммом 2278 активность ПТЭ повышается более слабо и только через 48 час. после заражения.

Как указывалось, активность ПТЭ в сильной степени зависит от присутствия Ca^{2+}. Было установлено, что оптимальная концентрация Ca^{2+} при определении активности ПТЭ составляла в условиях опыта 0,01 *M* (табл. 1). Связывание ионов Ca 0,005 *M* ЭДТА приводит к полному подавлению активности ПТЭ.

Таблица 1

Зависимость активности ПТЭ от содержания в ферментном растворе* ионов Ca

№№ п. п.	Количество Ca^{2+}	$D_{550 m\mu}$
	Зараженные клубни	
1	1 мл 0,02 *M* $CaCl_2$	0,570
2	1 мл 0,01 *M* $CaCl_2$	1,10
3	1 мл 0,001 *M* $CaCl_2$	0,715
4	Без добавления $CaCl_2$	0,195
5	То же + 2 мл 0,005 *M* ЭДТА	0,042
	Незараженные клубни	
6	1 мл 0,01 *M* $CaCl_2$	0,048

* Ферментный раствор: экстракт тканей клубней Любимец. 24 часа после заражения.

Результаты работы свидетельствуют о том, что ПТЭ является основным ферментом P. phytophthorum, вызывающим мацерацию тканей при заболевании картофеля черной ножкой; активность ПТЭ находится в прямой зависимости от степени вирулентности штаммов бактерии. Существенная роль ПТЭ в патогенезе заболевания, таким образом, несомненна. Однако должен быть особо отмечен тот факт, что повышение активности ПТЭ после заражения тканей восприимчивого сорта наблюдается, по нашим данным, гораздо позже, чем повышение активности протеиназ ([13]). Активность ПТЭ, следовательно, является только одним из факторов, обусловливающих патогенность бактерии.

Научно-исследовательский институт
картофельного хозяйства
Красково Московской обл.

Поступило
31 III 1969

ЦИТИРОВАННАЯ ЛИТЕРАТУРА

[1] D. F. Bateman, R. L. Millar, An. Rev. Phytopathol., 4, 119 (1966). [2] Н. А. Шишелова, Микробиология, 17, 3 (1948). [3] M. Gregg, Ann. Bot. N. S., 16, 62, 235 (1952). [4] A. F. Murant, R. K. Wood, Ann. Appl. Biol., 45, 635 (1957). [5] B. A. Friedman, M. J. Ceponis, Science, 129, 720 (1959). [6] М. В. Горленко, Хе Ли-юань, Вестн. Московск. унив., сер. VI, № 6 (1961). [7] B. A. Friedman, Phytopathol., 52, 4, 328 (1962). [8] D. Knösel, Zs. Pflanzenkrankh. u. Pflanzenschutz, 74, 3 (1967). [9] D. H. Lapwood, Ann. Bot. N. S., 21, 81, 167 (1957). [10] G. C. Papavizas, W. A. Ayers, Phytopathol., 55, 1, 111 (1965). [11] R. K. Horst, Phytopathol., 55, 8, 848 (1965). [12] W. K. Smith, J. Gen. Microbiol., 18, 1, 33 (1958). [13] И. И. Рагозина, Ю. И. Шнейдер, Д. В. Липсиц, ДАН, 184, № 1 (1969). [14] J. G. Hancock, Phytopathol., 56, 8, 975 (1966). [15] H. Neukom, Chimia (Schwitz.), 14, 165 (1960). [16] Т. К. Гапоненков, З. И. Проценко, Консервная и овощесуш. пром., 1, 29 (1963). [17] P. A. Albersheim, H. Neukom, H. Deul, Helv. chim. acta, 43, 5 (1960). [18] J. G. Hancock, M. E. Stanghellini, Canad. J. Bot., 46, 4, 405 (1968). [19] M. Dean, R. K. Wood, Neth. J. Pl. Path., 74 (Suppl. 1), 81 (1968). [20] J. G. Hancock, Phytopathol., 58, 1 (1968).

appearance of ametachromatic cells in cultured white cells from heterozygous carriers, compared to the more gradual disappearance of metachromasia in cells derived from the homozygous individuals, suggests that this temporal relation may prove to be a convenient way of distinguishing heterozygous from homozygous individuals. Quantitative studies on a larger number of families will be needed to substantiate these preliminary findings.

KATHLEEN M. FOLEY
B. SHANNON DANES
ALEXANDER G. BEARN
Division of Human Genetics, Department of Medicine, Cornell University Medical College, New York 10021

References and Notes

1. R. S. Krooth, G. A. Darlington, A. A. Velazquez, *Annu. Rev. Genet.* **2**, 141 (1968).
2. G. Brante, in *Cerebral Lipidoses*, J. N. Cumings, Ed. (Blackwell, Oxford, 1957).
3. U. Mittwoch, *Brit. J. Haemat.* **5**, 365 (1959).
4. A. Alder, *Deut. Arch. Klin. Med.* **183**, 372 (1939); W. A. Reilly, *Amer. J. Dis. Child.* **62**, 489 (1941).
5. B. S. Danes and A. G. Bearn, *Lancet* **1967-I**, 241 (1967).
6. ——, *J. Exp. Med.* **123**, 1 (1966).
7. M. E. Fedorko, unpublished.
8. Y. Rabinowitz, *Blood* **23**, 811 (1964).
9. L. Levintow and H. Eagle, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1962), vol. 5, p. 80.
10. E. H. Y. Chu and N. H. Giles, *Amer. J. Human Genet.* **11**, 63 (1959).
11. B. S. Danes and A. G. Bearn, *J. Exp. Med.* **126**, 509 (1967).
12. ——, *ibid.*, **124**, 1181 (1966).
13. Supported by a grant from The National Foundation. We thank Dr. George Jervis for referring patients to us and Sylvia Dillon for assistance.

7 March 1969

Potassium, Corticosterone, and Adrenocorticotropic Hormone Release in vitro

Abstract. *Incubation c*
sium increases adrenoco
pressed by the addition
are consistent with a pr
glands and suggest that
physial cell membrane t

EBREY, T.G. & CLAYTON, R.K. 1969

Phycomyces: Stimulus storage in light initiated reactions.

Science N.Y. 164 (3878):427, 3 graphs.

Microfilm no. 15614

Douglas and co-wor
proposed that the relea
amines from the adren
of vasopressin from t
physis is initiated by a
of the cell membrane. T
tion alters the permeabi
membrane allowing Ca+
cells. The increased co
intracellular Ca++ is th
activate a process leadi
release. The process has been called "stimulus-secretion coupling" (*1*). We have now obtained evidence to suggest that the release of adrenocorticotropic hormone (ACTH) from the adeno-

Table 1. Effect of a high concentration of K^+ on adrenocorticotropic hormone release in vitro. Results are expressed as adrenocorticotropic hormone content of incubating medium (KRB, High K^+-KRB), as a percentage of nonincubated pituitary halves (parentheses enclose 95 percent confidence limits). The medium was drawn off and replaced with fresh medium at 10, 30, and 90 minutes.

Incubation time (min)	ACTH release: KRB (%)	ACTH release: High K^+-KRB (%)
1–10	0.45(0.27–0.73)	2.92(2.37–3.58)
10–30	0.41(0.25–0.68)	4.04(3.33–4.91)
30–90	0.51(0.32–0.81)	7.41(5.92–9.27)

with glucose (KRB) (*3*), (ii) KRB containing a fivefold increase in K^+ (28 m*M*), isotonicity being maintained by alteration of the molarity of the NaCl, or (iii) KRB containing corticosterone (1 μg/ml). Solutions were gassed with 95 percent O_2 and 5 percent CO_2. Incubating media were assayed for adrenocorticotropic hormone content; the latter is expressed as a percentage of the content of the nonincubated halves. Parentheses enclose the 95 percent confidence limits (Table 1). All incubations were preceded by a 30-minute incubation in KRB; this medium was discarded. Exposure to a fivefold increase in K^+ significantly increased adrenocorticotropic hormone release.

We then investigated the effect of corticosterone on this augmented release of ACTH. The addition of corticosterone to the incubating medium significantly reduced the augmented release of ACTH from 13.40 (9.76 to 18.38) percent to 5.23 (4.01 to 6.83) percent, with a 90-minute incubation.

This effect of the high concentration of K^+ is not unique to the release of ACTH, since increased release of thyroid-stimulating hormone and luteinizing hormone have been reported under similar conditions (*4*, *5*). Indeed, Vale and Guillemin (*4*) have stated that increased concentrations of K^+ stimulate the release of ACTH in vitro. However they presented no data to support this statement.

We tentatively assume that this increased release of ACTH is secondary to a decrease in transmembrane potential. Such an effect could, however, have been due simply to a swelling of cells, such as occurs in muscle under similar conditions (*7*). But we found no significant change in weight when
ncubated in
increase in

physiological
rone depress
ced by high
the intact
ns of circu-
ress ACTH
gative feed-
been firmly
ing elements
are located.
suggest that
directly on
the adeno-
e.

/. MILLIGAN
G. CONRAD
C. M. BRANSON
Department of Physiology, Queen's University, Kingston, Ontario

References and Notes

1. W. W. Douglas, in *Neuroscretion*, F. Stutinsky, Ed. (Springer-Verlag, New York, 1967), pp. 178–190.
2. M. Saffran and A. V. Schally, *Endocrinology* **56**, 523 (1955).
3. W. W. Umbreit, R. H. Burris, J. F. Stauffer, in *Manometric Techniques* (Burgess, Minneapolis, 1964), p. 132.
4. W. Vale and R. Guillemin, *Experientia* **23**, 855 (1967).
5. M. H. Salmi and I. I. Geschwind, *Endocrinology* **82**, 225 (1968).
6. F. E. Yates, in *The Adrenal Cortex*, A. B. Eisenstein, Ed. (Little, Brown, Boston, 1967), pp. 133–183.
7. P. J. Boyle and E. J. Conway, *J. Physiol.* **100**, 1 (1941).
8. We thank A. Morris and Mrs. S. Reynolds for technical aid. Supported by Associated Medical Services Inc. and the CIBA Company Ltd., and by a grant from the Medical Research Council of Canada. J.K. is a Medical Research Council of Canada scholar.

6 December 1968

ceived and stored so that, when the *Phycomyces* is returned to room temperature, the light reaction will take place (*2*).

Phycomyces: Stimulus Storage in Light-Initiated Reactions

Abstract. *The sporangiophores of* Phycomyces blakesleeanus, *a unicellular fungus, increase their growth rate temporarily when given a symmetric light pulse. When a sporangiophore is cooled until normal growth stops, the light pulse can still be perceived, and, after the sporangiophore is warmed to room temperature, the normal light-growth response is observed. Thus stimulus information can be "stored" at low temperatures in this sensory system.*

The sporangiophores of the fungus *Phycomyces* show two classes of light responses (*1*). The first, phototropism, is a consequence of asymmetrical illumination, that is, a spatial inhomogeneity of light. The second, the light-growth response, is a consequence of a change in the intensity of symmetric illumination, that is, a temporal inhomogeneity of light. When light is arranged about the sporangiophore symmetrically (for instance bilaterally), and the light intensity is given a short (1 minute) steplike increase, then there is a temporary increase in the growth rate. If the increase in light intensity is maintained rather than cut off as in a pulse, then the growth rate will, nevertheless, return to normal. Thus, in continuous illumination, the growth rate is independent of the background light intensity.

One of the intermediate steps in the chain of events between stimulus and response can be stabilized at a given temperature. Thus, when a sporangiophore is cooled and growth stops, a light stimulus can nevertheless be perceived and stored so that, when the *Phycomyces* is returned to room temperature, the light reaction will take place (*2*).

Phycomyces blakesleeanus was grown on potato dextrose agar. The sporangiophores develop through four distinct stages (*1*). Our experiments were done with stage 4 sporangiophores. The rate of growth of the sporangiophores was measured with a microscope and graticule modified according to Dennison (*3*).

To obtain symmetrical illumination the sporangiophores were illuminated with blue light by two lamps placed opposite each other. Red light was used as the "working" safe light.

The best type of device to cool the air around the sporangiophores consisted of a glass enclosure for the sporangiophore around which ice could be placed. It was found that if the temperature changes were to affect the growth rate reversibly, the temperature decrease must be gradual and the ice was found to give a sufficiently slow rate of cooling.

In these experiments we observed the light-growth response rather than phototropism, because unwanted movements due to temperature changes were less pronounced for the light-growth measurements. The temperature of the cooled sporangiophores was between 1° to 5°C. At these temperatures the sporangiophores, which normally grow at about 1 mm per hour, stopped growing.

Several combinations of light and temperature were used. Figure 1 shows a typical light-growth response, that is, one without any temperature changes made. The light-growth response (Fig. 1A) was elicited by a moderate pulse increase of light intensity (*4*). Without cooling, the time from when the light was turned on until the maximum growth rate was achieved was about 4.5 minutes. When a 5-minute bilateral pulse was given 5 minutes after the start of a 12-minute cold period, there was no light-growth response. Then the cooling device was removed so that the sporangiophore was exposed to air at room temperature, and a normal light-growth response was observed. These experiments were repeated several times.

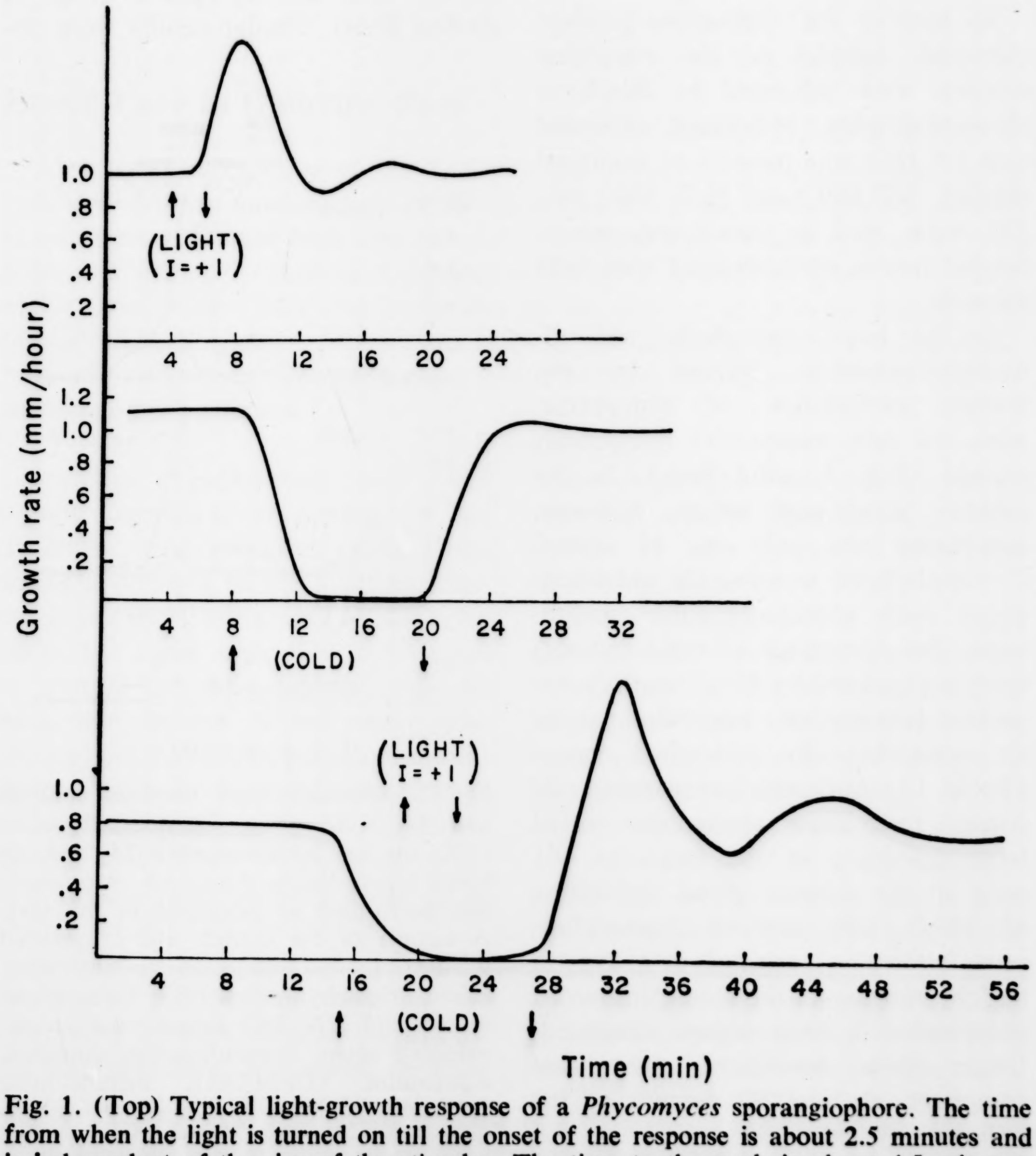


Fig. 1. (Top) Typical light-growth response of a *Phycomyces* sporangiophore. The time from when the light is turned on till the onset of the response is about 2.5 minutes and is independent of the size of the stimulus. The time to the peak is about 4.5 minutes. (Center) Effect of gently cooling a *Phycomyces* sporangiophore and then allowing it to return to room temperature. (Bottom) Stimulus storage in a *Phycomyces* sporangiophore. After the sporangiophore was cooled, and it had stopped growing, a 5-minute light pulse is given. After 8 minutes the sporangiophore is returned to room temperature and a light-growth response is observed.

THOMAS G. EBREY*
R. K. CLAYTON

Section of Genetics, Development, and Physiology, Cornell University, Ithaca, New York 14850

cross-react (*11*). The present observations suggest that luciferin from fishes and *Cypridina*, whether derived vicariously or synthesized independently, may be more widely distributed as a common substrate than heretofore believed.

YATA HANEDA
Yokosuka City Museum, Yokosuka, Japan
FREDERICK I. TSUJI
Department of Biophysics and Microbiology, University of Pittsburgh, and Veterans Hospital, Pittsburgh, Pennsylvania
NOBORU SUGIYAMA
Department of Chemistry, Tokyo Kyoiku University, Tokyo, Japan

References and Notes

1. K. Kato, *Zool. Mag. Tokyo* **57**, 195 (1947); T. Iwai and H. Asano, *Sci. Rep. Yokosuka City Mus.* **3**, 5 (1958).
2. Y. Haneda and F. H. Johnson, *Proc. Nat. Acad. Sci. U.S.* **44**, 127 (1958); *J. Morphol.* **110**, 187 (1962).
3. Y. Haneda, *Sci. Rep. Yokosuka City Mus.* **11**, 1 (1965).
4. ——, F. H. Johnson, E. H.-C. Sie, *Biol. Bull.* **115**, 336 (1958); *Sci. Rep. Yokosuka City Mus.* **4**, 13 (1959).
5. F. H. Johnson, Y. Haneda, E. H.-C. Sie, *Science* **132**, 422 (1960).
6. F. H. Johnson, N. Sugiyama, O. Shimomura, Y. Saiga, Y. Haneda, *Proc. Nat. Acad. Sci. U.S.* **47**, 486 (1961).
7. E. H.-C. Sie, W. D. McElroy, F. H. Johnson, Y. Haneda, *Arch. Biochem. Biophys.* **93**, 286 (1961).
8. F. I. Tsuii, *ibid.* **59**, 452 (1955); F. I. Tsuii and R. Sowinski, *J. Cell. Comp. Physiol.* **58**, 125 (1961).
9. F. I. Tsuji, Y. Haneda, N. Sugiyama, in preparation.
10. F. I. Tsuji and Y. Haneda, in *Bioluminescence in Progress*, F. H. Johnson and Y. Haneda, Eds. (Princeton Univ. Press, Princeton, N.J., 1966), pp. 137–149; *Sci. Rep. Yokosuka City Mus.* **13**, 12 (1967).
11. Y. Haneda, *Rec. Oceanogr. Works Jap.* **1**, 103 (1953).
12. We are grateful to Dr. T. Abe of the Tokaiku Fisheries Institute of Tokyo who identified the Philippine apogonids. Supported by NSF grant GF-274 and the Japan Society for the Promotion of Science under the United States–Japan Cooperative Science Program. Publication No. 153 from the Department of Biophysics and Microbiology, University of Pittsburgh. Drs. R. L. Bolin and J. B. Buck offered helpful suggestions.

15 April 1969

Schizophyllum commune: Gene Controlling Induced Haploid Fruiting

Abstract. *A single gene,* fis+, *is essential for induced haploid fruiting in* Schizophyllum commune. *It appears to segregate independently of the incompatibility factors.*

In tetrapolar Basidiomycetes, such as *Schizophyllum commune*, reproductive morphogenesis can be envisioned as consisting of two kinds of development. (i) The series of reactions—nuclear migration, formation of clamp-connections, synchronous nuclear division, and so forth—that establishes the dikaryon, and (ii) the maturative changes in the dikaryotic mycelium that culminate in meiosis and sporulation in the fruiting bodies. The process of dikaryosis and its control by the incompatibility factors have been studied extensively (*1*), but the cellular mechanisms that underlie the development of fruiting bodies in dikaryotic mycelia are largely unknown. It is also uncertain whether the incompatibility factors play an essential role in the fruiting process.

Although the formation of fruiting bodies normally occurs as a corollary of dikaryosis, fruiting structures may develop from haploid mycelia under certain circumstances (*2–4*). A recent study showed that haploid fruiting is inducible by an exogenous chemical agent or agents (*4*). The inducing factor was first isolated from mycelial extracts of the imperfect fungus *Hormodendrum cladosporoides* (*Cladosporium*) and was later obtained from fruiting bodies of *S. commune* and *Agaricus bisporus*. Methods of extraction, partial purification, and a biological assay were established for the inducing substance or substances.

An initial survey showed that very few homokaryons of *S. commune* would respond to the fruiting-inducing substance or substances (FIS). These observations raised the question as to the extent and nature of the genetic contribution to FIS-induced fruiting. We now report evidence of a single gene that functions in FIS-induction of haploid fruiting in *Schizophyllum commune*.

When an FIS-inducible strain was crossed with a compatible, noninducible homokaryon and a number of the resulting offspring were exposed to FIS, inducibility and noninducibility showed a 1:1 segregation ($P = .3$ to $.5$ in cross 1 of Table 1). Each monosporus mycelium was tested in triplicate, and the inducible cultures usually fruited by the 5th day after the application of FIS to the margins of the 4-day old colonies. The pattern of segregation indicated a single-gene difference among the two classes of progeny. The hypothesis of a single gene with two alleles, henceforth symbolized *fis*+ and *fis*−, was tested in three additional crosses (Table 1).

If neither of the mated strains carried the *fis*+ allele, none of the progeny would be expected to respond to FIS. On the other hand, when both compatible homokaryons possess the *fis*+ allele, all of their progeny would be expected to fruit following exposure to FIS. Both of these expectations were realized. Inducibility by FIS can thus be added to the several genetic factors previously demonstrated to be important in the expression of haploid fruiting in *S. commune* (*2, 5*).

The *fis* gene has segregated independently of the incompatibility factors in all tests conducted thus far (for example, $P = .3$ to $.5$ in cross 1 of Table 1). It is thus possible in compatible crosses between FIS-inducible and FIS-noninducible homokaryons to associate the *fis*+ allele with the incompatibility factors in any desired combinations.

THOMAS J. LEONARD*
JOHN R. RAPER
Department of Biology, Harvard University, Cambridge, Massachusetts 02138

Table 1. Mode of inheritance of FIS-induced haploid fruiting.

Mated strains	Offspring tested (No.)	*fis*+	*fis*−
A41 B41 fis+ × *A51 B51 fis*−	84	38	46
A41 B41 fis+ × *A51 B51 fis*+	30	30	0
A41 B41 fis+ × *A43 B43 fis*+	30	30	0
A41 B41 fis− × *A51 B51 fis*−	20	0	20

References and Notes

1. J. R. Raper, *Genetics of Sexuality in Higher Fungi* (Ronald, New York, 1966).
2. ——— and G. S. Krongelb, *Mycologia* **50**, 707 (1958).
3. D. J. Niederpruem, H. Hobbs, L. Henry, *J. Bacteriol.* **86**, 1300 (1964).
4. T. J. Leonard and S. Dick, *Proc. Nat. Acad. Sci. U.S.* **59**, 745 (1968); T. J. Leonard, thesis, Indiana University (1967).
5. S. L. Mishkin, thesis, Indiana University (1967).
6. T.J.L. was an NIH postdoctoral research fellow, 1967–68.
* Present address: Department of Botany, University of Kentucky, Lexington 40506.
17 April 1969

Cyclophosphamide: Effect on Experimental Allergic Encephalomyelitis in Lewis Rats

Abstract. *Lewis rats sensitized to spinal cord in adjuvant and exhibiting advanced clinical paralytic signs of experimental allergic encephalomyelitis were treated with cyclophosphamide (5 milligrams per kilogram) daily. Of 30 treated animals, 21 recovered rapidly and appeared clinically well within 7 to 12 days. This immunosuppressive agent may prove therapeutically useful in the treatment of autoimmune diseases.*

The capacity of cytotoxic drugs to suppress antibody synthesis, delayed-type hypersensitivity, and allergic tissue injury elicited by a variety of antigenic stimuli is well established (*1*). Optimum immunosuppression usually has required administration of the cytotoxic agent at the time of or soon after antigenic stimulation; that is, well in advance of the time when the immune response in question would have otherwise occurred (*1*). This prerequisite for immunosuppression has severely restricted the use of most cytotoxic drugs in treatment of immunol

Experimental allergic e
litis (EAE) is a promisin
tem for the study of imm
tors implicated in central
tem disease of animals an
Complete inhibition of E
reported in rats sensitiz
cord and treated daily wi
phamide for 17 days afte
(*4, 5*).

In our study (*5*),
rats, equivalent results
when injections of this c
layed until the 9th day a
tion, that is, several da
appearance of paralysis i
mals. Furthermore, rats
then treated daily or 5 ... for 17 days after sensitization did not develop EAE for as many as 112 days (*5, 6*). Thus treatment with cyclophosphamide initiated after onset of EAE might exert a beneficial effect on the course of the disease. In that Lewis rats regularly develop a severe and often fatal form of EAE, they can be used for critical testing of any therapeutic action of a drug (*7, 8*). We now report striking improvement and the return to clinical well-being of Lewis rats with EAE treated with cyclophosphamide (CY) for 7 to 12 days after onset of paralytic signs.

Under light ether anesthesia male rats 7 to 10 weeks of age were sensitized to guinea pig (Hartley) spinal cord in adjuvant (H37Rv or BCG) by injection into the skin of the back (*9*). The animals then were placed in different treatment groups at random, housed in groups of eight in stainless steel cages and given free access to food pellets and water. Sensitized rats were observed daily for clinical neurologic signs of EAE—ataxic gait, weakness or frank paralysis of hind legs, "cord" bladder, incontinence of urine, and fecal impaction (*8*). Paralytic signs appeared in all animals 12 to 18 days after sensitization. On the day when first paralyzed, each rat was transferred to an individual cage for the duration of each experiment. Lewis rats become

onset of paralysis. Other control rats were not treated; they were left in individual cages and simply observed. All rats were killed 22 to 24 days after they were sensitized. Brains and spinal cords from these animals, as well as those that died before this time, were fixed in Formalin and prepared for histological examination (*9*). A minimum of three blocks of brain and four blocks of spinal cord from each rat was embedded in paraffin and sectioned; the sections were stained with hematoxylin and eosin. Microscopic lesions were graded according to an arbitrary scale of 0 to +++ (*11*).

The mean number of days after sensitization when cyclophosphamide and saline injections began was 14.5 (range 12 to 18) and 14.4 (range 12 to 18), respectively. Mean number of days after onset of paralytic signs when either type of treatment was started was 2.1 (range 1 to 5) for both groups (*12*).

Of 30 rats with EAE treated with the drug (Table 1), only three of the survivors still had slight ataxia or questionable hind-leg weakness 22 to 24 days after sensitization. The 21 rats
s appeared
ert by this
usually be-
ost treated
ll strength
previously
appearance
ary incon-
cal impac-
and return
body hair.
28 saline-
which were
from pre-
in this rat
. Many of
vived until
ization still
e weakness
of the hind legs and "cord" bladder.

Four of the six deaths in the CY-treated group occurred after only 1 to 4 days of treatment, perhaps before the drug could exert adequate effects. An additional death occurred after 9 days of treatment in a rat that had recovered after 4 days of treatment and had remained clinically well for 4 days. He then developed an unsteady gait on day 23 and was found dead the next day. The sixth rat was killed on day 20 when he suddenly appeared ill and moribund after dramatic improvement. Urine and blood cultures yielded *Proteus* sp. Severe urinary tract infection and bacteremia would appear to account for his clinical deterioration.

An experiment with seven rats sensitized and treated with the drug after onset of paralysis is not included in

Table 1. Clinical status of Lewis rats with EAE after cyclophosphamide treatment.

Treatment	Rats in group (No.)	Rats (No.)		
		Dead	Paralytic signs*	Clinically well†
Cyclophosphamide	30	6	3	21
Saline	28	13	12	3
Not treated	22	11	11	0

* Unsteady gait, hindleg weakness or paraplegia, and "cord" bladder. † No detectable clinical neurological abnormalities; appearance indistinguishable from normal rats. Probabilities from chi-square calculations with Yates correction for small numbers: CY versus saline <.001; CY versus not-treated <.001; CY versus saline plus not-treated <.001.

LEONARD, T.J. & RAPER, J.R. 1969

Schizophyllum commune: gene controlling induced haploid fruiting.

Science, N.Y., 165 (3889):190.

Microfilm no. 15615

Toxins in Plant Disease: Structure and Mode of Action

Toxins may cause disease symptoms by inhibiting enzymes or changing the permeability of membranes.

Lowell D. Owens

The concept that plant pathogens cause disease by producing toxic substances dates back about a century. Evidence for its general validity, however, has accumulated only recently. The difficulty has been that most plant disease symptoms are the result of a complex interplay of several factors. Some pathogens, in addition to producing one or more toxins, may also excrete enzymes which degrade cell walls, causing tissue disorganization; they may destroy plant hormones, causing abnormal growth of the host; or they may physically block the water-conducting vessels of the host by their prolific growth or by production of viscous polysaccharides. Because of this complexity, proof of the role of a toxin as one of several interacting factors causing a disease symptom is often difficult to obtain, even though the presence of toxic substances in cultures of most plant pathogens is easily demonstrated. Despite these problems, our growing knowledge of the biochemistry of symptom causation clearly establishes the role of toxins as a dominant one in most plant diseases.

In plant pathology a toxin is generally defined as a nonenzymic substance which injures plant cells or disrupts their metabolism. Plant toxins are of lower molecular weight than are animal toxins, and most do not induce antibody formation when injected into animals. Although the structure and mode of action of relatively few toxins have been thoroughly investigated, sufficient information is now available to permit an integrated presentation of the subject. I will discuss here only those toxins produced by pathogens for which data on their chemical nature and mode of action are available. This includes two antimetabolites, several host-specific polypepetides, and a number of miscellaneous compounds of low molecular weight. Pathogen-produced enzymes and plant hormones will not be considered.

The author is research microbiologist, U.S. Soils Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, Beltsville, Maryland.

Antimetabolite Action of Wildfire Toxin

Wildfire disease in tobacco is a much cited example in which one symptom is caused by an antimetabolite synthesized by the pathogen. The phytopathogenic bacterium *Pseudomonas tabaci* produces a toxin which causes chlorosis in the form of a halo around the necrotic locus of infection (*1*). The toxin, isolated from cultures of the bacterium, is capable of inducing chlorosis in many plant species other than tobacco (*2*).

Woolley and associates (*3*) first isolated wildfire toxin and proposed a tentative structure, α-lactylamino-β-hydroxy-ε-aminopimelic acid lactone (*4*), a modified dipeptide lactone. Later, an attempted synthesis led them to postulate that the correct structure was an unspecified structural isomer of the one first proposed (*5*).

The mode of action of wildfire toxin was investigated concurrently by Braun (*6*) using sterile culture filtrates of *P. tabaci* which contained the toxin and the unicellular green alga *Chlorella vulgaris* as a test organism. Growth of this alga was inhibited by the toxin, but the inhibition could be prevented by adding extracts of liver or yeast. L-Methionine was found to be the factor capable of preventing toxicity to chlorella, thus suggesting that the toxin was an antimetabolite of L-methionine. In support of this reasoning, Braun further demonstrated that a competitive relationship existed between the amount of toxin in the medium and the amount of L-methionine required to prevent growth inhibition. However, attempts to prevent chlorosis in tobacco leaves by adding L-methionine failed.

Additional support for the methionine antimetabolite theory was sought with a newly discovered structural analog of methionine, methionine sulfoximine (MSO) (Fig. 1), an animal convulsant isolated from bleached wheat flour. Methionine sulfoximine caused chlorotic halos when injected into tobacco leaves, and it inhibited growth of chlorella (*1*). As in the case of wildfire toxin, MSO toxicity to chlorella, but not to tobacco, was prevented by simultaneous addition of L- methionine. The parallel toxicities exhibited by the toxin and MSO were ascribed to the hypothesis that both were
structu
of L-n
suppor
tants o
to MS
toxin (
The
Woolle
the un
eases.
gists to
may d
hosts by
importa
time. N
wildfire
metabo
eral in
structure proposed for wildfire toxin bore little structural resemblance to methionine. (ii) Methionine failed to prevent toxicity to tobacco leaves by either wildfire toxin or MSO. (iii) The parallel toxicities of toxin and MSO and resistance of chlorella mutants to both compounds, while suggesting similar mode of action, did not necessarily implicate methionine antagonism. As Braun (*1*) pointed out, the mechanism of MSO toxicity was not fully understood, since MSO inhibition of bacterial growth could be reversed equally well by either methionine (*7*) or glutamine (*8*). Further, MSO had been reported to inhibit a glutamine-synthesizing enzyme from *Staphylococcus aureus* and sheep brain (*9*). In recognizing this ambiguity Braun (*1*) tested L-glutamine

OWENS, L.D.
Toxins in plant disease: stru
Science, N.Y., 165 (3888):18-
89 ref. U.S. Soils Lab., U
Microfilm no.
156

for its ability to prevent MSO toxicity to chlorella; none was observed. Finally, the possibility existed that methionine prevented MSO and toxin inhibition in chlorella by preventing uptake of these two compounds, as was later found to be the case for MSO entry into animal cells (*10*), and not by competing for a metabolic site within the cell.

Following this initial work, which demonstrated the similarities in biological activities of MSO and wildfire toxin, evidence continued to accumulate that MSO was an inhibitor of glutamine synthesis and that amelioration of MSO toxicity in animals by methionine was due to indirect effects (*11*). Also, rather high concentrations of glutamine were shown to prevent MSO toxicity to wheat embryos (*12*). Thus, MSO became recognized as an antimetabolite of glutamic acid and not of methionine in some organisms.

The question of the mode of action of wildfire toxin was reopened by Sin-
when
into
sions
(*13*).
that
bited
rat
This

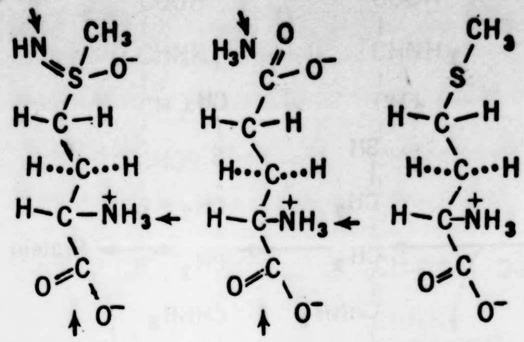
$+ P_i$

ostu-
nter-

syn-
that
peti-
tive and noncompetitive inhibition with respect to glutamic acid (*13*). Ronzio and Meister (*15*) have attempted to explain a similar mixed-type inhibition of glutamine synthetase by MSO by postulating a two-step inhibition process. The rate of the first step, presumably binding of MSO to the catalytic site, is competitive with glutamate. The second step, formation of MSO phosphate which remains tightly bound to the enzyme, is not reversible by glutamate, and hence, is noncompetitive. The parallel inhibition kinetics of toxin and MSO toward glutamine synthetase would suggest a similar mechanism of inhibition by the two compounds.

1969
: structure and mode of action.
8):18-25. [4 diag., 1 table,
ab., U.S.D.A., Beltsville, Md.]
616

The structural analogy of MSO and glutamate (*15*) is shown in Fig. 1. Apparently, the oxo and imino func-



Methionine sulfoximine   Glutamate   Methionine

Fig. 1. Possible orientations of methionine sulfoximine, glutamate, and ammonia on glutamine synthetase. Arrows indicate possible sites of attachment. [Taken from Ronzio and Meister (*15*).] The structure of methionine is shown to illustrate the lack of structural analogy to the other two compounds regarding the γ-carbon functional groups.

tions of MSO are necessary for attachment to the enzyme binding sites normally occupied by the γ-carboxyl group of glutamate and ammonia. Lack of these functions in methionine explains why it neither inhibits glutamine synthetase nor protects the enzyme against MSO inhibition (*11*).

Sinden and Durbin further related this in vitro enzyme inhibition to cause of symptoms in tobacco. They found that concentrations of toxin or MSO inhibitory to glutamine synthetase in vitro caused chlorosis in tobacco leaves (*13*). Treated leaves also suffered a buildup of ammonia to concentrations about seven times that of untreated leaves. Both chlorosis and ammonia buildup were prevented by simultaneous injection of high concentrations of L-glutamine into the leaves. This probably means that either glutamine prevented cellular uptake of the toxic compounds or that it protected glutamine synthetase from being inhibited. Either action would account for the lower ammonia levels.

On the basis of their results, Sinden and Durbin propose that inhibition of glutamine synthetase may be the primary action of tobacco wildfire toxin (*13*). They further suggested that the chlorosis symptom is more likely caused by toxic amounts of ammonia or other intermediates of nitrate metabolism rather than by induced deficiency of glutamine. The ammonia presumably arises from continued nitrate reduction by the plant while one of its main pathways for ammonia incorporation into organic compounds is blocked.

One remaining question is the structure of wildfire toxin. Sinden and Durbin (*16*) recently reported that the toxin contains a threonine moiety, which was not originally found by Woolley *et al.* (*3*).

Pseudomonas tabaci is only one of several species of this genus that cause diseases in various plants. Do all phytotoxic pseudomonads produce the same toxin? Sinden and Durbin (*16*) have isolated a toxin from *P. coronafaciens* (causing halo blight of cereals) cultures which is identical to wildfire toxin. On the other hand, they have obtained considerable evidence to suggest that the halo-inducing toxins from *P. phaseolicola* and *P. tomato* (bean and tomato pathogens) are different from wildfire toxin (*17*). Also, DeVay, Sinden, and co-workers (*18*) have isolated a polypeptide toxin from cultures of the peach tree pathogen, *P. syringae* [= *P. morsprunorum* (*19*)].

Antimetabolite Action of Rhizobium Toxin

Rhizobial-induced chlorosis in soybeans is the second example of a disease thought to be caused by an antimetabolite produced by the pathogen. This disease arises from a rather unique association between two organisms, an association that is both symbiotic and pathogenic. Certain strains of the legume root-nodule bacterium *Rhizobium japonicum* fix nitrogen in an apparently normal fashion and simultaneously synthesize a toxin which induces chlorosis in the new leaf growth of the soybean host plant (*20*). This toxin has been isolated from the nodules and chlorotic leaves of diseased plants (*20*) and from the culture medium of the bacterium (*21*).

The toxin from *R. japonicum*, "rhizobitoxine" (*22*), is not host-specific. At low concentrations it causes chlorosis in seedlings of many plants (*20*, *23*). Because the toxin apparently causes all of the disease symptoms and because toxin production in the nodule is essential to disease expression (*20*), rhizobitoxine may be classified as a primary disease determinant. This disease is defined as those aspects of the association that are not part of the normal symbiotic relationship and that are clearly deleterious to the host.

The precise structure of the toxin has yet to be determined. It is known to be a basic sulfur-containing amino acid. Upon desulfurization, it yields a nontoxic amino acid (termed unknown *Y*)

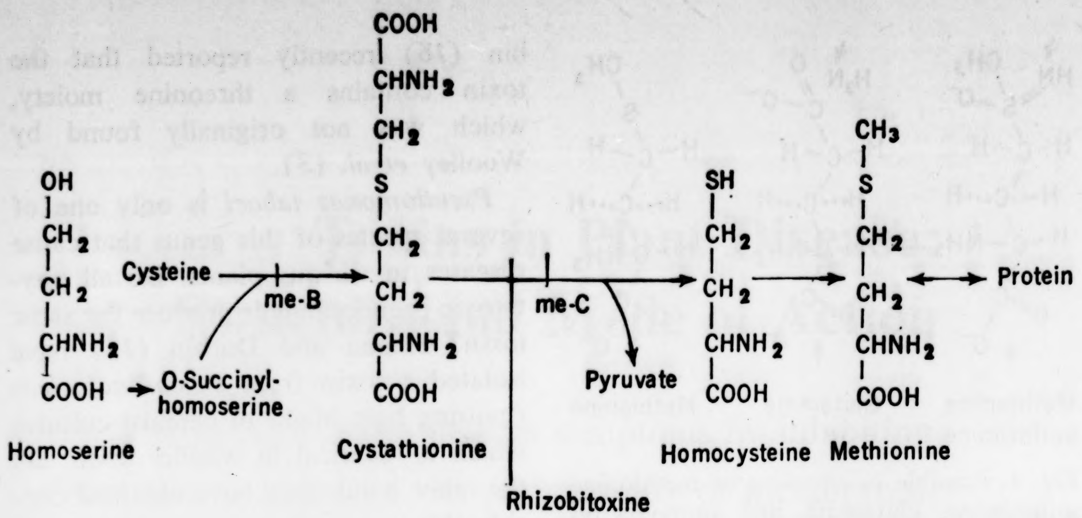


Fig. 2. Pathway of methionine biosynthesis in *Salmonella typhimurium*. β-Cystathionase catalyzes the cleavage of cystathionine to form homocysteine and is inhibited by rhizobitoxine. Metabolic blocks of methionine-requiring mutants, me-B and me-C, of *Salmonella* are indicated.

that also occurs naturally in soybean nodules and in *R. japonicum* cultures (*24*). Unknown *Y* has been tentatively identified as an ether derivative of homoserine (*24*), R-O-CH_2-CH_2-CH (NH_2)-COOH, where R is a three-carbon moiety containing an amino and a hydroxyl group (*25*).

Several lines of evidence suggest that the toxin is composed of two molecules of unknown *Y* joined through a thioether linkage (*24*). This interpretation is attractive considering mode of action hypotheses, as will be discussed later, but other interpretations have not been ruled out.

A logical approach to determining the mode of action became possible once the toxin was known to be a sulfur-containing amino acid. One would obviously suspect toxin interference with the metabolism of one of the naturally occurring sulfur-containing amino acids. Initially, attempts to prevent chlorosis in sorghum seedlings by adding methionine to the root solution along with the toxin gave ambiguous results. Chlorosis was apparently reduced but at methionine concentrations that bordered on being toxic (*26*).

Salmonella typhimurium proved to be a much more suitable organism for investigating the mechanism of action. Growth of this bacterium was inhibited by low concentrations of rhizobitoxine (0.25 mmole/liter), and the inhibition could be completely prevented by simultaneously adding small amounts of L-methionine or its immediate precursor, homocysteine (*22*). The toxin inhibition was not ameliorated, however, by adding earlier precursors of methionine, namely, cystathionine and homoserine (Fig. 2).

The possibility that cystathionine was simply not entering the cells was eliminated by performing the same experiment with a methionine-requiring mutant (me-B in Fig. 2) that can utilize cystathionine as a source of methionine. The same results were obtained as with the wild type.

These experiments suggested that the toxin was inhibiting the enzymatic cleavage of cystathionine to form homocysteine, thereby inducing a deficiency of methionine. We found this to be true in experiments with the isolated cleavage enzyme (*22*). The apparent K_m of β-cystathionase for its substrate cystathionine was 0.36 mmole/liter while the apparent K_i for the toxin was 0.02 μmole/liter. Thus, the enzyme affinity for the toxin was over 10,000 times that for its substrate. The toxin inhibition was mixed type.

It was concluded that the primary site of action of rhizobitoxine in *Salmonella* was the inhibition of β-cystathionase. The deficiency of methionine induced thereby resulted in decreased rates of protein synthesis and, consequently, decreased growth.

The question as to whether this same mechanism was operative in plants was investigated with a strain of the unicellular green alga *Chlorella pyrenoidosa* that is extremely sensitive to the toxin (*26*). Addition of L-methionine to the culture medium along with the toxin provided partial relief of growth inhibition.

The effect of rhizobitoxine on β-cystathionase isolated from spinach leaves has been determined (*27*). As in the case of *Salmonella*, the toxin proved to be a potent inhibitor of this enzyme. Thus, rhizobitoxine is an antimetabolite of cystathionine in both *Salmonella* and spinach. A spinach seedling develops chlorosis from 1 μg of toxin added to its root solution.

Thus, there is considerable evidence that the mechanism of rhizobitoxine action in higher plants is the same as in *Salmonella* (*27*). Although the pathway of methionine biosynthesis in plants has not been fully elucidated, there is evidence to suggest it is similar to that in *Salmonella* (*28*). β-Cystathionase activity has been observed in cell-free extracts of a number of higher plants, thus suggesting its involvement in methionine biosynthesis (*29*). It seems likely, therefore, that β-cystathionase inhibition is a major, if not the sole, site of rhizobitoxine action in higher plants and hence the basis of this disease.

What structural features of rhizobitoxine account for its antimetabolite action against cystathionine? To answer this question we need to know both what part of the cystathionine molecule (Fig. 3) is involved in binding to the β-cleavage enzyme and where the sulfur substituent is located in the toxin molecule. Neither are known. However, a thioether substituent on C-4 of unknown *Y* would provide analogy to the four-carbon moiety of cystathionine, while the same substituent on C-3 would resemble the three-carbon moiety (Fig. 3).

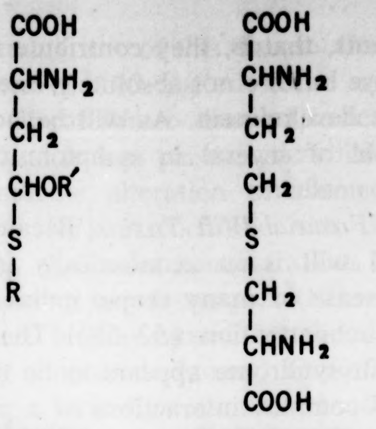
Host-Specific Polypeptide Toxins

Several phytopathogenic fungi are known to produce polypeptide toxins. These are highly specific compounds that only affect the host of the pathogen, and they are primary determinants of the diseases in which they are involved (*30*). They are smaller than animal polypeptide toxins and resemble certain polypeptide antibiotics and animal hormones, both in size and structure (*31*). The apparent similarities among these various classes of biologically active polypeptides may even extend to their modes of action. Certain members of each class are postulated to exert their biological influence primarily by altering the permeability of cell membranes (*32*).

Helminthosporium victoriae causes blight only in Victoria variety of oats or in its derivative cultivars (*30*). Victoria was imported from South America and used extensively to breed smut and crown rust resistance into North American oat varieties. Shortly after the release of these cultivars, in about 1955, "Victoria blight" became epidemic throughout the United States.

The primary determinant of this disease, a potent toxin called victorin, was discovered by Meehan and Murphy in 1947 (*33*). Pringle and Braun isolated and partially characterized the toxin as a polypeptide derivative with a molecular weight between 800 and 2000 (Table 1) (*34*). Mild alkali treatment cleaved the toxin into a biologically inactive polypeptide moiety composed of five or six common amino acids and a nitrogen-containing sesquiterpine moiety called victoxinine (*30, 35*). Victoxinine is 1/7500 as toxic as the intact toxin, is not host-specific, and is produced in a free form by both pathogenic and nonpathogenic isolates of *H. victoriae*. Its role as a disease determinant is therefore doubtful, except as an integral part of the toxin.

The cleaved peptide moiety of victorin is nontoxic and, when added to solutions of the intact toxin, reduces its toxicity (*36*). This suggests that the peptide is competing for toxin receptor sites and, therefore, that toxin specificity is conferred by its peptide moiety. The finding that sulfite also reduces toxicity without affecting the toxin is some evidence for the involvement of carbonyl groups in the receptor site (*30*). According to a preliminary report, bisulfite and semicarbazide also protect tissue against toxin-induced electrolyte loss into a bathing solution (*37*).



Rhizobitoxine Cystathionine

Fig. 3. A possible structure of rhizobitoxine illustrating analogy to the four-carbon moiety of cystathionine.

Although many secondary effects of the toxin from *H. victoriae* have been reported, the primary effect is now postulated to be an alteration in cell membrane permeability. Wheeler and Black (*38*) showed that toxin-treated or infected tissue from a susceptible oat variety rapidly begins to lose electrolytes into a bathing solution. This electrolyte loss commences within 5 minutes after toxin treatment, has a low temperature coefficient characteristic of a physical process, and is induced by 1/50 the concentration required to increase respiration. Tissue from resistant oat varieties was not affected, which shows that these are host-specific and not general effects.

Further evidence for the membrane-alteration theory was obtained by Samaddar and Scheffer (*39*) with plant cell protoplasts, that is, cells stripped of their cell walls by enzymic digestion but retaining an intact cell membrane. Protoplasts from susceptible oat tissue quickly stopped protoplasmic streaming and burst within 1 hour after toxin treatment. Protoplasts from resistant oats were little affected.

Luke and co-workers (*40*) have obtained electron-microscopic evidence for selective toxicity toward cell membranes. The endoplasmic reticulum, nuclear envelope, and mitochondrial membranes were more resistant to toxin damage. These findings have been corroborated by studies showing that neither succinoxidase activity (*41*), respiratory control (*42*), nor permeability (*38*) of mitochondria isolated from susceptible oat tissue was affected by toxin treatment.

Helminthosporium carbonum is closely related to *H. victoriae* but pathogenic to certain corn hybrids. The fungus produces a host-specific toxin in pure culture, which has been isolated and crystallized by Pringle and Scheffer (*43*) (Table 1). Acid hydrolysis yielded amino acids or amino-containing degradation products. This fungus also produces a second toxic polypeptide, carbtoxinine. It is much less toxic (Table 1) and lacks host specificity. Unlike victoxinine, there is no indication that carbtoxinine is a part of the toxin from *H. carbonum*. Its role as a disease de-

Table 1. Properties of polypeptide and related plant toxins.

Toxin	Host-specific	Host	Lowest toxic concentration (μg/ml)	Chemical nature	Elemental proportions, or known products of hydrolysis
Helminthosporium victoriae toxin (victorin)	Yes	Oats	0.01	Polypeptide derivative, ninhydrin negative	Aspartate, glutamate, valine, glycine, leucine and/or isoleucine victoxinine
Victoxinine* (*H. victoriae*)	No	Oats	75	*N*-containing sesquiterpene	$C_{17}H_{29}NO$
Helminthosporium carbonum toxin	Yes	Corn	0.5†	Polypeptide, ninhydrin negative	Amino compounds
Carbtoxinine (*H. carbonum*)	No	Corn	25†	Polypeptide, ninhydrin positive	Amino compounds
Periconia circinata					
Toxin A	Yes	Grain sorghums	0.1	Polypeptide	Six alanine, four aspartate two glutamate, two serine
Toxin B	Yes		?	Polypeptide	?
Corynebacterium sepidonicum toxin	No	Potato	500	Glycopeptide	$C_{18}H_{98}O_{48}N$; MW21,450; threonine, serine, glycine, alanine, lysine, two methionine, glucose, mannose, and two unidentified sugars
Corynebacterium michiganense toxins	No	Tomato	400	Glycopeptides	I: $C_{42}H_{82}O_{40}N$; MW129,700 II: $C_{478}H_{940}O_{440}N$; MW>200,000 III: $C_{27}H_{52}O_{25}N$; MW35,280 All contain six amino acids and four sugars
Colletotrichum fuscum toxin (colletotin)	No	Digitalis	60	Glycopeptide ?	Glucose, galactose, mannose; peptide moiety ?

* Occurs naturally in free form and also as a constituent of *H. victoriae* toxin. † Concentration causing one-third to one-half inhibition.

terminant is questionable. Corn tissue treated with the toxin from *H. carbonum* shows none of the secondary metabolic disturbances observed with the toxin from *H. victoriae* (*44*). Dark CO_2 fixation appears to be stimulated (*45*).

Periconia circinata is the cause of "milo disease" in certain grain sorghums. In 1948 Leukel (*46*) discovered that a host-specific toxin was involved in milo disease. Two host-specific polypeptide toxins, termed A and B, were subsequently isolated from culture filtrates by Pringle and Scheffer (*47*) (Table 1). Toxin A, the major toxin, (molecular weight <2000) may possess a nonamino acid moiety. It induces metabolic changes in susceptible corn tissue in a fashion resembling that of the toxin from *H. victoriae* (*35*).

Glycopeptide Toxins

Most polysaccharides excreted by certain phytopathogenic bacteria cause wilting of the host plant by physical obstruction of water-conducting vessels (*48*). Several polysaccharide toxins possess a polypeptide moiety which increases the possibility that their mechanism of action is not merely physical obstruction.

Four glycopeptide wilt toxins have been isolated from two species of *Corynebacterium* and partially characterized (Table 1) by Rai and Strobel (*49*). They proposed that the toxins play a major role in production of disease symptoms (*49*). Several lines of evidence, including autoradiography of cells treated with labeled toxin and electron-microscopic observations, suggest that the mode of action involves membrane changes and not plugging of vascular elements.

Colletotin is a nondialyzable toxin produced by the phytopathogenic fungus *Colletotrichum fuscum*. Goodman (*50*) partially purified the toxin and obtained good evidence for a polysaccharide moiety. Proof of the existence of a suspected peptide moiety is still lacking. The toxin appears to act by causing disintegration of leaf mesophyll and palisade tissue (*51*).

Other Toxins

The structures of several plant toxins produced by fungi are shown in Fig. 4. Most are secondary disease determinants, that is, they contribute to a disease but are not absolutely essential for its development. As will be noted, the role of several in symptom causation is unclear.

Fusarial Wilt Toxins. Because fusarial wilt is an economically important disease of many crops, it has received much attention (*52–59*). The fusarial wilt syndrome appears to be the result of complex interactions of a pathogen-produced plant hormone, several toxins, and toxic enzymes. The syndrome usually includes epinasty, plugging and browning of xylem vessels, necrosis, wilt, and ultimately death. Various toxins have been implicated, including fusaric acid, dehydrofusaric acid, α-picolinic acid, phytonivein, novarubin and several related compounds, and lycomarasmin—but not all in the same diseased plant.

Fusaric acid (Fig. 4) is produced by cultures of many species of *Fusarium* (*53*), and this acid has been detected in tomato (*54, 55*) and wilted cotton (*56*). It forms a weak chelate with metal ions (*60*) and inhibits rice catalase by forming a complex with its heme-Fe prosthetic group (*61*). The inhibition depends upon the presence of the carboxyl group in the fusaric acid molecule and can be reversed by addition of Fe^{3+}. The toxin does not inhibit enzymes lacking bound metals. The actual concentrations of fusaric acid in the host tissue need to be known before metal-enzyme complexing can be established as a mechanism of action.

Fusaric acid also caused electrolyte loss, wilting of tomato cuttings (*57, 53*), and changes in cell permeability of rice tissue (*54*) and of *Rhoeo discolor* epidermis (*62*). Some cells are affected by as little as $10^{-9}M$ fusaric acid (*55*). Electrolyte leakage also occurred from fusaria-infected tomato leaves (*63*). α-Picolinic acid (Fig. 4) and dehydrofusaric acid, the *n*-butylene analog of fusaric acid, apparently have toxic properties and mechanisms similar to those of fusaric acid (*62, 64*).

Phytonivein was isolated from cultures of the watermelon wilt organism, *F. oxysporum* f. *niveum* (*58, 65*). It has an empirical formula $C_{29}H_{46}O_2$ and many properties of steroids. At low concentrations it permanently wilts watermelon seedlings.

Kern and Naef-Roth have isolated four phytotoxic pigments (naphthazarin derivatives) from various cultures of *Fusarium solani* and from diseased pea (*66*). The structure of one, novarubin, is shown in Fig. 4 (*67*). The severity of disease expression in pea from infection or from toxin treatment was diminished by adding certain metal ions, providing indirect but not conclusive evidence for the involvement of these pigments in symptom causation (*66*). The four toxins presumably cause wilting in seedlings of various plants by affecting the water permeability of the cell membranes.

Lycomarasmin is a toxin produced by *Fusarium oxysporum* f. *lycopersici*. The suggested role of lycomarasmin as a secondary determinant of tomato wilt disease is a matter of controversy (*52, 53, 57*). Woolley (*68*) postulated lycomarasmin to be the modified tripeptide shown in Fig. 4. The synthesized compound was as toxic as the natural product (*69*). Lycomarasmin causes wilting of tomato cuttings (*53*). Its toxicity is increased tenfold by the presence of iron, with which it forms a weak chelate (*53, 70*), but it is detoxified by other metal ions (*58*). In the presence of iron, lycomarasmin causes increased transpiration of tomato cuttings and increased water permeability of epidermal strippings from *Rhoeo discolor* leaves (*58*). When *Tradescantia* leaves were treated with lycomarasmin, protoplasmic streaming ceased within 30 minutes (*52*). Damage to cell membranes appears to be an early effect.

Blast disease is one of the two principal diseases of rice. Certain symptoms—leaf desiccation and yellowing and stunting of seedlings—are attributed to two toxins produced in cultures of the fungal pathogen *Piricularia oryzae* and in diseased plants (*71*). One toxin was identified as α-picolinic acid (Fig. 4). The other, piricularin, was obtained crystalline by Tamari and Kaji (*71*), who proposed a tentative empirical formula of $C_{18}H_{14}N_2O_3$. It exhibited some specificity for susceptible varieties, and 1 μg/ml enhanced respiration in rice seedlings.

Helminthosporium leaf spot, the other principal disease of rice, is caused by the fungus *Cochliobolus miyabeanus* (*Helminthosporium oryzae*). Nakamura and co-workers (*72*) have isolated the toxin ophiobolin (Fig. 4) from cultures of the fungus and have obtained evidence for its production in diseased plants (*73*). Treatment of rice leaves with 10 μg of ophiobolin per milliliter causes necrotic spots resembling those of the disease and inhibition of rice seedling root elongation.

Ophiobolin induces production of

phenolic compounds in rice leaves (*72*), a phenomenon also observed with some other plant toxins. Subsequent oxidation and polymerization of these phenolics produces the brown pigments apparent in necrotic spots.

A related fungus, *Cochliobolus sativus*, causes common root rot of cereals, particularly wheat and barley. Fungal attack of barley seedlings causes necrosis and stunting, effects that can be reproduced by treatment with helminthosporal. This toxin, isolated from culture filtrates of *C. sativus*, is a sesquiterpenoid dialdehyde (Fig. 4) (*74*). It is probably produced by the fungus during invasion.

Helminthosporal inhibits both electron transfer and oxidative phosphorylation in isolated mitochondria (*75*). The site of electron transfer inhibition appears to be between flavoprotein dehydrogenase(s) and cytochrome c. Inhibition of these respiratory processes may constitute the mechanism of phytotoxicity, since seedlings treated with helminthosporal suffer respiration inhibition. It is interesting that a reduced analog of helminthosporal, namely helminthosporol, has gibberellin-like growth-promoting properties.

Alternaria solani causes early blight disease of various Solanaceae such as potatoes and tobacco. Alternaric acid, a hemiquinone derivative (Fig. 4) (*76*), has been isolated from culture filtrates of the fungus (*77*) and is thought to be a secondary determinant of this disease (*57, 58*). Alternaric acid causes severe wilt of seedlings of many plants at 5 μg/ml (*53, 77, 78*).

Leaf and stem blight in zinnia, sunflower, and marigold has been attributed to the fungus *Alternaria zinniae*. Several symptoms in zinnia, including stem withering, chlorosis, and leaf-tip curling, appear to be caused by zinniol, a toxin isolated from culture filtrates of the fungus (*79*). The structure of zinniol is shown in Fig. 4 (*80*). At a concentration of 500 μg/ml it causes rapid loss of pigment from cut red beet stems and drastic shriveling of the stems, which suggests that cell membrane damage is an early effect (*79*).

Ascochitine is another example of a phytotoxic hemiquinone derivative. It was isolated from culture filtrates of *Ascochyta fabae*, the causal fungus of brown spot disease of broad bean, and characterized by Oku and Nakanishi (Fig. 4) (*81*). Application of 10 μg of ascochitine per milliliter to broad bean coleoptiles causes the brown necrotic spots characteristic of this disease. Concentrations of 100 μg/ml cause epidermal cells of *Rhoeo* discolor to leak pigment and to lose plasmolytic ability, which indicate alteration of the cell membrane (*81*). Ascochitine was detoxified by equimolar concentrations of Fe^{3+}, yielding a red complex (*82*).

Chestnut blight, caused by the accidentally imported fungus *Endothia parasitica*, virtually eliminated the American chestnut tree about 40 years ago. Gäuman and co-workers have isolated two toxins from cultures of the pathogen which are highly active in causing changes in water permeability of cells of *Rhoeo discolor* (*83*). Diaporthin, $C_{30}H_{18}O_{10}$, is active at $10^{-11}M$, while skyrin, a dianthraquinone (Fig. 4), is active at $10^{-6}M$ (*84*). Both are about equally toxic to tomato shoots. Although diaporthin induces symptoms of chestnut blight, apparently neither toxin has been isolated from diseased tissue.

Problems and Prospects

We are now aware of the wide variety of compounds that cause disease symptoms; we know the chemical structure of a number of them, and we understand the mode of action of several. Still, we are unable to describe, in molecular detail, the mechanisms by which most toxins damage plant cells. With the few exceptions noted, the

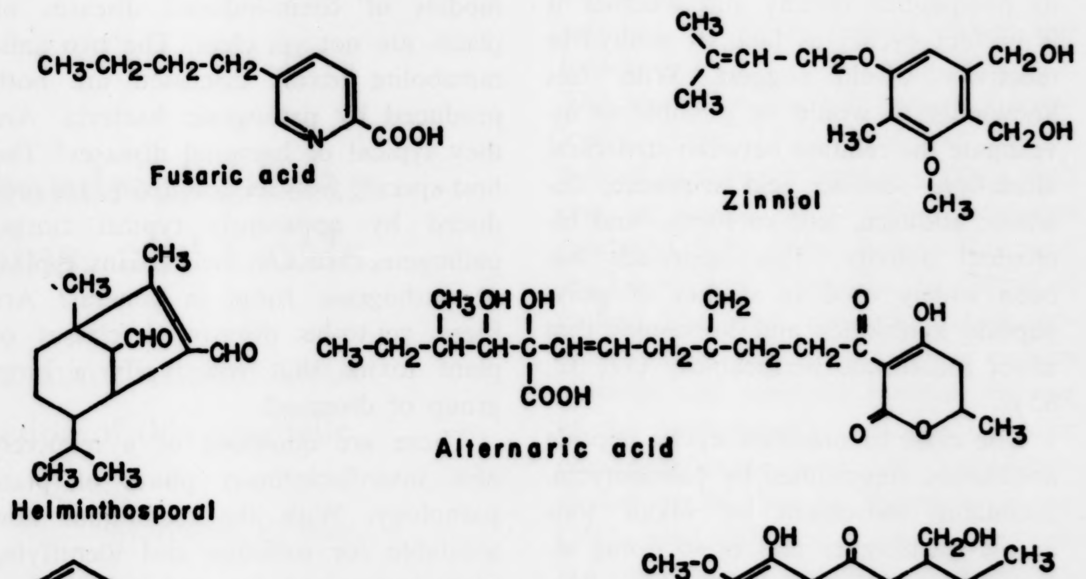


Fig. 4. Structures of pathogen-produced plant toxins: fusaric acid (*53*), zinniol (*80*), helminthosporal (*74*), alternaric acid (*76*), α-picolinic acid (*71*), novarubin (*67*), ascochitine (*81*), ophiobolin (*72*), skyrin (*83*), and lycomarasmin (*68*).

most common earliest sign of toxin damage to cells is an alteration in water or ion permeability of the cytoplasmic membrane. Does this mean that each of these chemically diverse compounds reacts with a specific membrane component to alter its permeability characteristics? Is there any physical relationship between the water permeability and selective metabolite permeability characteristics of membranes? Is the membrane, in fact, the primary site of action?

The most interesting of toxins in regard to these questions are the host-specific polypeptides. The one most studied, the toxin from *Helminthosporium victoriae*, causes membrane damage within minutes at very low concentrations and exhibits specificity for the cytoplasmic membrane. One would like to know the amino acid sequence of its polypeptide moiety and whether it is in fact cyclic, as lack of ninhydrin reactivity would suggest. With this knowledge, it would be possible to investigate the relation between structural alterations—amino acid sequence, deletion, addition, and so forth—and biological activity. This approach has been widely used in studies of polypeptide antibiotics and hormones that affect membrane permeability (*31, 32, 85*).

One class of modified cyclic peptide antibiotics, represented by valinomycin, facilitates movement of alkali ions across membranes and in so doing alters their semipermeability (*31, 86*). The enniatins are members of this class synthesized by various species of *Fusarium*. Although enniatins injure tomato cuttings at low concentrations, their role in fusarial wilt has not been established (*55*). Studies relating possible capacities of polypeptide plant toxins to form ion complexes and their solubilities in lipids might be fruitful.

The mechanisms by which other toxins affecting membrane permeability operate may be more amenable to study than the polypeptide toxins. They are usually easier to isolate, more stable, and easier to label with a radioisotope. Use of labeled toxins should indicate whether or not the cell membrane is the primary site of action. Analogs of toxins of low molecular weight would be easier to prepare for determining the structural requirements for toxicity and the nature of the receptor site. Some of this work has been done with fusaric acid (*61*) and the toxin from *H. victoriae* (*30*).

Investigations of the way in which toxins affect membrane transport need not be limited by the complexities of intact cells. Membrane sacs (or vesicles), reconstituted from disrupted cytoplasmic membranes of bacteria, have been used to study selective transport of amino acids (*87*). They offer a simplified system which retains the selective transport properties of the intact cell membrane. Artificial membranes have also been proved useful for studying antibiotic-facilitated transport of ions (*86*), for determining membrane constituents necessary for antibiotic action of certain polyenes (*88*), and for predicting the effect of unsaturated hydrocarbon chains on water permeability of bean root membranes (*89*).

In the investigation of any subject one attempts to identify model systems from which he may generalize. The models of toxin-induced diseases of plants are not yet clear. The two antimetabolite toxins discussed are both produced by pathogenic bacteria. Are they typical of bacterial diseases? The host-specific polypeptide toxins are produced by apparently typical fungal pathogens, but are their toxins typical of pathogenic fungi in general? Are there yet-to-be discovered classes of plant toxins that will typify a large group of diseases?

These are questions of a relatively new interdisciplinary phase of plant pathology. With the techniques now available for isolating and identifying plant toxins and for studying their reactions with living cells, we can expect many exciting developments as we come to further understand plant disease.

References and Notes

1. A. C. Braun, *Phytopathology* **45**, 659 (1955).
2. E. E. Clayton, *J. Agr. Res.* **48**, 411 (1934).
3. D. W. Woolley, R. B. Pringle, A. C. Braun, *J. Biol. Chem.* **197**, 409 (1952); D. W. Woolley, G. Schaffner, A. C. Braun, *ibid.* **198**, 807 (1952).
4. D. W. Woolley, *ibid.* **215**, 485 (1955).
5. ———, in *Plant Pathology, Problems and Progress*, C. S. Holton, G. W. Fischer, R. W. Fulton, H. Hart, S. E. A. McCallan, Eds. (Univ. of Wisconsin Press, Madison, 1959), p. 130; J. M. Stewart, *J. Amer. Chem. Soc.* **83**, 435 (1961).
6. A. C. Braun, *Proc. Nat. Acad. Sci. U.S.* **36**, 423 (1950).
7. G. W. Newell and W. W. Carman, *Fed. Proc.* **9**, 209 (1950).
8. J. G. Heathcote and J. Pace, *Nature* **166**, 353 (1950).
9. W. Shive and G. G. Skinner, in *Metabolic Inhibitors*, R. M. Hochster and J. H. Quastel, Eds. (Academic Press, New York, 1963), vol. 1, p. 1.
10. C. Lamar, Jr., and O. Z. Sellinger, *Biochem. Pharmacol.* **14**, 489 (1965).
11. O. Z. Sellinger and P. Weiler, Jr., *ibid.* **12**, 989 (1963); O. Z. Sellinger and J. M. Azcurra, *Fed. Proc.* **27**, 756 (1968).
12. J. J. C. Hinton and T. Moran, *Brit. J. Nutr.* **11**, 323 (1957).
13. S. L. Sinden and R. D. Durbin, *Nature* **219**, 379 (1968).
14. C. Lamar, S. L. Sinden, R. D. Durbin, *Mol. Pharmacol.*, in press.
15. R. A. Ronzio and A. Meister, *Proc. Nat. Acad. Sci. U.S.* **59**, 164 (1968).
16. S. L. Sinden and R. D. Durbin, *Phytopathology* **58**, 1067 (1968).
17. ———, *ibid.* **59**, 249 (1969).
18. J. E. DeVay, F. L. Lukezic, S. L. Sinden, H. English, D. L. Coplin, *ibid.* **58**, 95 (1968); S. L. Sinden and J. E. DeVay, *ibid.* **57**, 102 (1967).
19. J. E. Cross, *ibid.* **58**, 1203 (1968).
20. L. D. Owens and D. A. Wright, *Plant Physiol.* **40**, 927 (1965).
21. ———, *ibid.* p. 931.
22. L. D. Owens, S. Guggenheim, J. L. Hilton, *Biochim. Biophys. Acta* **158**, 219 (1968).
23. H. W. Johnson, U. M. Means, F. E. Clark, *Nature* **183**, 308 (1959).
24. L. D. Owens and J. F. Thompson, *Bacteriol. Proc.* (1967), p. 19.
25. ———, K. Biemann, unpublished.
26. L. D. Owens, unpublished.
27. J. Giovanelli, L. D. Owens, S. H. Mudd, unpublished.
28. J. F. Thompson, *Annu. Rev. Plant Physiol.* **18**, 59 (1967).
29. J. Giovanelli and S. H. Mudd, *Plant Physiol.* **41** (Suppl.), xiii (1966).
30. For a comprehensive review of host-specific plant toxins, see R. B. Pringle and R. P. Scheffer, *Annu. Rev. Phytopathol.* **2**, 133 (1964); and for more recent developments, see R. P. Scheffer and R. B. Pringle, in *The Dynamic Role of Molecular Constituents in Plant-Parasite Interactions*, C. J. Mirocha and I. Uritani, Eds. (American Phytopathological Soc., St. Paul, Minn., 1967), p. 217.
31. E. Schroeder and K. Luke, *The Peptides* (Academic Press, New York, 1966), vol. 2.
32. O. Hechter, in *Mechanisms of Hormone Action*, P. Karlson, Ed. (Academic Press, New York, 1965); E. J. Modest, G. E. Foley, S. Farber, in *Metabolic Inhibitors*, R. M. Hochster and J. H. Quastel, Eds. (Academic Press, New York, 1963) vol. 1; H. B. Woodruff and I. M. Miller, *ibid.*, vol. 2, p. 23.
33. F. Meehan and H. C. Murphy, *Science* **106**, 270 (1947).
34. R. B. Pringle and A. C. Braun, *Nature* **181**, 1205 (1958).
35. R. B. Pringle, paper presented at the 1st International Congress of Plant Pathology, London, 1968, and personal communication.
36. R. P. Scheffer and R. B. Pringle, *Phytopathology* **54**, 832 (1964).
37. K. R. Samaddar, *ibid.* **58**, 1065 (1968).
38. H. Wheeler and H. S. Black, *Science* **137**, 983 (1962); ———, *Amer. J. Bot.* **50**, 686 (1963).
39. K. R. Samaddar and R. P. Scheffer, *Plant Physiol.* **43**, 21 (1968).
40. H. H. Luke, H. E. Warmke, P. Hanchey, *Phytopathology* **56**, 1178 (1966); P. Hanchey, H. Luke, H. Wheeler, *Amer. J. Bot.* **55**, 53 (1968).
41. R. B. Grimm and H. Wheeler, *Amer. J. Bot.* **53**, 436 (1963); L. R. Krupka, *ibid.* **49**, 587 (1959); R. P. Scheffer and R. B. Pringle, *ibid.* **53**, 465 (1963).
42. H. Wheeler and P. J. Hanchey, *Science* **154**, 1569 (1966).
43. R. B. Pringle and R. P. Scheffer, *Phytopathology* **57**, 1169 (1967).
44. M. S. Kuo and R. P. Scheffer, *ibid.*, p. 817.
45. ———, *ibid.* **58**, 1056 (1968).
46. R. W. Leukel, *J. Agr. Res.* **77**, 201 (1948).
47. R. B. Pringle and R. P. Scheffer, *Phytopathology* **56**, 1149 (1966); ———, *ibid.* **57**, 530 (1967).
48. R. N. Goodman, Z. Kiraly, M. Zaitlin, *The Biochemistry and Physiology of Infectious Plant Disease* (Van Nostrand, Princeton, N.J., 1967), pp. 286 and 322.
49. P. V. Rai and G. A. Strobel, *Phytopathology* **59**, 53 (1969); G. A. Strobel, *Plant Physiol.* **42**, 1433 (1967).
50. R. N. Goodman, *Phytopathol. Z.* **37**, 187 (1959).
51. S. Lewis and R. N. Goodman, *Phytopathology* **52**, 1273 (1963).
52. A. E. Dimond, *Annu. Rev. Plant Physiol.* **6**, 329 (1955).
53. E. Gäumann, *Endeavour* **13**, 198 (1954); for structure, see T. Yabutta, K. Kanbe, T. Hayashi, *J. Agr. Chem. Soc. Japan* **10**, 1056 (1934).

54. E. Gäumann, *Phytopathology* 47, 342 (1957).
55. R. K. S. Wood, *Physiological Plant Pathology* (Blackwell, Oxford, 1967).
56. T. S. Sadasivan, in *Recent Advances in Botany* (9th International Botanical Congress, Montreal, 1959), vol. 2, p. 1021.
57. H. Wheeler and H. H. Luke, *Annu. Rev. Microbiol.* 17, 223 (1963).
58. R. A. Ludwig, in *Plant Pathology*, J. G. Horsfall and A. E. Dimond, Eds. (Academic Press, New York, ed. 2, 1960), p. 315.
59. S. Nishimura, *Trans. Tottori Soc. Agr. Sci.* 14, 4 (1962).
60. S. Malini, *Phytopathol. Z.* 57, 221 (1966).
61. K. Tamari and J. Kai, *Bull. Fac. Agr. Niigata Univ. Japan* 6, 1 (1954).
62. E. Bachman, *Phytopathol. Z.* 27, 255 (1956).
63. R. P. Collins and R. P. Scheffer, *Phytopathology* 48, 349 (1958).
64. B. D. Sanwal, in *Recent Advances in Botany* (9th International Botanical Congress, Montreal, 1959), vol. 2, p. 1012.
65. I. Hiroe and S. Nishimura, *J. Agr. Chem. Soc. Japan* 30, 528 (1956).
66. H. Kern and S. Naef-Roth, *Phytopathol. Z.* 53, 45 (1965); ———, *ibid.* 57, 289 (1966).
67. ———, *ibid.* 60, 316 (1967).
68. D. W. Woolley, *J. Biol. Chem.* 176, 1291 (1948).
69. ———, *ibid.*, p. 1299.
70. P. E. Waggoner and A. E. Dimond, *Phytopathology* 43, 281 (1953).
71. K. Tamari and J. Kaji, *Bull. Fac. Agr. Niigata Univ. Japan* 5, 33 (1954); K. Tamari, N. Ogasawara, J Kaji, *Proc. Int. Bot. Congr., Edinburgh*, 10, 80 (1964).
72. H. Oku, in *The Dynamic Role of Molecular Constituents in Plant-Parasite Interactions*, C. J. Mirocha and I. Uritani, Eds. (American Phytopathological Soc., St. Paul, Minn., 1967), p. 237; for structure, see S. Nozoe, K. Morisaki, K. Tsuda, I. Iitaka, N. Takahashi, S. Tamura, K. Ishibashi, M. Shirasaka, *J. Amer. Chem. Soc.* 87, 4968 (1965).
73. M. Nakamura and H. Oku, *Ann. Takamine Lab.* 12, 266 (1960).
74. P. deMayo, E. Y. Spencer, R. W. White, *Can. J. Chem.* 41, 2996 (1963).
75. E. Taniguchi and G. A. White, *Biochem. Biophys. Res. Commun.* 28, 879, (1967).
76. J. R. Bartels-Kieth, *J. Chem. Soc.* 1662 (1960).
77. P. W. Brian, G. W. Elson, H. G. Hemming, C. W. Unwin, J. M. Wright, *Nature* 164, 534 (1949).
78. E. Gäumann, S. Naef-Roth, P. Reusser, A. Ammann, *Phytopathol. Z.* 19, 160 (1952).
79. G. A. White and A. N. Starratt, *Can. J. Bot.* 45, 2087 (1967).
80. A. N. Starratt, *Can. J. Chem.* 46, 767 (1968).
81. H. Oku and H. Nakanishi, *Phytopathol. Z.* 55, 1 (1966).
82. ———, *Phytopathology* 53, 1321 (1963).
83. A. E. Boller, E. Gäumann, E. Hardegger, F. Kugler, S. Naef-Roth, M. Rosner, *Helv. Chim. Act.* 40, 875 (1957); E. Gäumann and S. Naef-Roth, *Pflanzenschutz Ber.* 19, 9 (1957).
84. E. Gäumann and W. Obrist, *Phytopathol. Z.* 37, 145 (1960).
85. T. Wieland, *Science* 159, 946 (1968); A. W. Bernheimer, *ibid.*, p. 847.
86. M. M. Shemyakin, Y. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, A. M. Shkrob, I. I. Mikhaleva, A. V. Evstratov, G. G. Malenkov, *Biochem. Biophys. Res. Commun.* 29, 834 (1967); B. C. Pressman, E. J. Harris, W. S. Jagger, J. H. Johnson, *Proc. Nat. Acad. Sci. U.S.* 58, 1949 (1967).
87. H. R. Kaback and E. R. Stadtman, *Proc. Nat. Acad. Sci. U.S.* 55, 920 (1966); H. R. Kaback, *J. Biol. Chem.* 243, 3711 (1968).
88. G. Sessa and G. Weismann, *Biochim. Biophys. Acta* 135, 416 (1967).
89. P. J. C. Kuiper, *Science* 143, 690 (1964).

Mitochondrial DNA: Advances, Problems, and Goals

Studies of size and structure of mitochondrial DNA relate to biogenesis and function of this organelle.

Margit M. K. Nass

The concerted efforts of biologists and biochemists have led to the discovery that deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are distinct components of cytoplasmic organelles, in particular of mitochondria and chloroplasts. Various problems pertaining to the heredity and biogenesis of mitochondria and chloroplasts, and to the structure, function, and synthesis of nucleic acids and proteins in these organelles, have been reviewed (*1–8*). It is characteristic of our present age of rapid scientific developments, involving exponentially growing numbers of individuals and teams, that the pertinent literature on mitochondrial DNA, which was in its infancy in the early 1960's (*8*), far exceeds the scope of an article in these pages today. I restrict this discussion, therefore, to the more recent advances in the structure and biochemistry of mitochondrial DNA and present some of the problems we confront at the present level of knowledge and insight (*9*).

The author is assistant professor of therapeutic research, University of Pennsylvania School of Medicine, Philadelphia 19104.

Structure and Size of DNA Molecules

The most common conformation of mitochondrial DNA of multicellular animal cells is a double-stranded circle with a perimeter of 4.7 to 5.5 microns (*10, 11*) corresponding to a molecular weight of 9×10^6 to 10×10^6 daltons. Circular mitochondrial DNA has been described in most classes of vertebrates, including man (*12–15*), birds (*16*), and amphibians (*17*); it was found in sea urchin (*18*) and as a minor DNA component of mitochondria in yeast (*19*). The circular DNA molecules observed in the electron microscope consist of a mixture of highly twisted forms and loosely twisted, or open, types (Fig. 1). The twisted structure is typical of covalently closed DNA, and the loosely twisted forms may represent DNA with one or more single-strand scissions (nicked DNA). Other factors, however, are known that determine the degree of coiling. The DNA molecules can be separated on gradients consisting of cesium chloride and ethidium bromide by use of the principle that less dye (at high concentrations) binds to covalently closed circles than to nicked or linear DNA, and different buoyant densities are imparted to the various molecules regardless of base composition (*12*).

The very small differences in length that have been observed for mitochondrial DNA's from various cell types are frequently due to technical factors in the hands of different investigators. Generally, the ionic strength of the medium (hypophase) upon which the DNA molecules are spread as a DNA-protein monolayer affects the molecular lengths significantly (*20*). The molecules may shorten by about 10 percent at ionic strengths above 0.1 mole per liter, compared with distilled water as a hypophase. The existence of true size differences was shown by spreading different mitochondrial DNA's together as mixtures. The measurements of size obtained from a mixture of mitochondrial DNA from mouse fibroblasts (L cells) and from chicken liver followed a bimodal distribution with peak categories (4.7 to 4.8 μ and 5.1 to 5.2 μ, respectively) corresponding to the size observed when each DNA was spread individually. The existence of small size

PRIX DE REVIENT

Le prix de revient varie beaucoup suivant plusieurs facteurs mais, en règle générale, il est plus bas dans une production rationnelle et dans les cultures irriguées. Suivant une enquête faite en 1966 en culture irriguée, le prix de revient variait de 129 à 190 piastres pour 1 kilogramme de coton-graine dans la région de TCHOUKOUROVA. Dans la région d'EGÉE, il oscillait entre 127 et 170 piastres tandis que, dans le département d'Antalya, il était en moyenne de 130 piastres. Il était supérieur de 10 à 15 % dans les cultures en sec. Dans les fermes annexes des Instituts de Recherches et des Stations du Coton, il variait entre 89,47 et 225 piastres pour la campagne 1967/68.

Les prix d'achat du coton-graine des Coopératives figurent au tableau 14.

1969

DJAVADI, F.H.S.

Recherche de Verticillium dahliae Kleb. dans les semences de Cotonniers d'Iran. [Search for V. dahliae in Iranian Cotton seed.]

Coton Fibr. trop., 24 (3):315-316. [16 ref. Engl. Span. summ.]

Microfilm no. 15617

RECHERCHE DE *Verticillium dahliae* Kleb. DANS LES SEMENCES DE COTONNIERS D'IRAN

par

FERIDOUM H. S. DJAVADI

Docteur-Ingénieur

Verticillium dahliae Kleb., parasite polyphage, induit le dépérissement de nombreuses plantes cultivées et en particulier le « wilt » du cotonnier dans de nombreuses régions de culture à travers le monde, et, particulièrement, en Iran.

La transmission et la dissémination du *Verticillium* par les semences du cotonnier, sujet de nombreux travaux contradictoires, pose un problème qui n'a pas été parfaitement résolu.

REVUE BIBLIOGRAPHIQUE

TAUBENHAUS (1936, 1937) isole le parasite de l'intérieur des graines, mais formule ensuite (d'après RUDOLPH et coll. 1944) des doutes sur ses propres résultats. YUEN et TAN (1958) isolent le parasite à partir des semences délintées à l'acide sulfurique et avancent des pourcentages d'infection allant jusqu'à 40 %. Pour reprendre l'expression de MASSENOT (1964), ces résultats semblent extraordinaires. Par ailleurs, ALLEN (1951) inocule par injection des semences de cotonnier et isole le pathogène 8 mois plus tard ; il en conclut que celui-ci peut se transmettre par les semences. Par contre, MILES (1934), CHEO (1944) ainsi que RUDOLPH et coll. (1944) (malgré 5 années d'expériences précises sur des semences provenant de plantes malades) ne peuvent isoler le champignon de l'intérieur des semences et considèrent que les graines des plantes malades ne sont pas contaminées.

La contamination externe fait l'objet des mêmes controverses. HANSFORD (1937), CHEN et coll. (1964), EVANS et coll. (1966) isolent le champignon à partir des fibres adhérant aux téguments des semences ; WICKENS (1948), IN (1958), COGNÉE (1963) pensent que *Verticillium* est transmissible par les semences, sans toutefois en apporter de preuves précises, tandis que RUDOLPH et coll. (loc. cit.), SOLOVEVA et coll. (1948) estiment que ce mode de transmission est négligeable.

Les techniques d'isolement classiques (méthodes d'Ulster) n'ont pas révélé la présence de *V. dahliae* à l'extérieur ou à l'intérieur des semences de cotonniers de provenance iranienne (DJAVADI, 1969).

Les résultats suivants ont été obtenus en appliquant des techniques spéciales.

TECHNIQUES

Nous avons utilisé deux techniques spéciales pour isoler *V. dahliae* : la méthode des dilutions et les observations microscopiques.

1. Méthode des dilutions (de NADAKAVUKAREN et coll., 1959, modifiée).

100 semences sont immergées dans 100 ml d'eau distillée stérile et sont agitées mécaniquement pendant 30 mn. Des parties aliquotes du surnageant (10 ml) sont introduites dans des fioles d'Erlenmeyer contenant 90 ml d'un milieu gélosé à 7,5 ‰ maintenu liquide à 42 °C, renfermant 10 mg de streptomycine. Juste avant l'introduction du surnageant, on y ajoute 0,5 ml d'éthanol absolu. Le contenu de chaque fiole est réparti dans cinq boites de Pétri. Celles-ci sont incubées à l'obscurité à 27 °C.

2. Observations microscopiques (EVANS et coll., 1966)

Des lots de 50 semences d'un échantillon donné sont lavés 5 fois avec 20 ml d'eau distillée stérile. La première eau de lavage contient 1 % de Tween 20 facilitant le mouillage des fibres attachées à la surface des semences (« lint »). Les eaux de lavage sont collectées et mélangées.

20 ml de la solution obtenue sont filtrés sur une membrane Millipore (diamètre des pores : 0,45 μ). La membrane une fois sèche est découpée en carrés d'un centimètre de côté ; chaque carré est monté sur une lame porte-objet et traité avec quelques gouttes de bleu-coton au lactophénol. Les membranes Millipore deviennent transparentes au contact du colorant ce qui permet l'observation microscopique directe des spores et surtout des microsclérotes de *Verticillium dahliae*.

RÉSULTAT

La méthode proposée par NADAKAVUKAREN permet la croissance de nombreux *Aspergillus*, *Fusarium* et *Alternaria* sur le milieu de culture et ne semble pas très spécifique du *Verticillium*. Toutefois, des microsclérotes introduits artificiellement dans l'eau de lavage des semences (environ 10 microsclérotes/ml d'eau de lavage) donnent naissance à des colonies identifiables. De même, la méthode d'EVANS et coll. permet une bonne observation sur les membranes Millipore des microsclérotes, quand ils sont présents.

Nous n'avons pas pu détecter la présence du champignon sur plus de 9 000 semences analysées par les différentes méthodes ; nous sommes amenés à penser que la transmission du *Verticillium dahliae* par les semences est négligeable dans le cas des cotonniers iraniens. Bien entendu, des observations étendues sur plusieurs années seront nécessaires avant d'affirmer entièrement ces résultats.

CONCLUSION

Nos expériences d'analyse de la mycoflore par la méthode d'ULSTER modifiée n'ont pas mis en évidence *Verticillium dahliae* comme contaminant des semences. De même, les méthodes particulières employées n'ont permis de déceler la présence de *Verticillium* ni à l'intérieur ni à l'extérieur des graines. Nous pensons, en conséquence, que l'infection interne des semences iraniennes de cotonnier est improbable.

BIBLIOGRAPHIE

ALLEN R.M., 1951. — *Plt Dis. Reptr.*, 35, 11-12.

CHEN C.T., S.S. CHEN et J.Y. WANG, 1964. — *Acta phytopath. Sin.*, 7, 135-142 (d'après : *Rev. appl. Mycol.*, 1965, 44, 2149).

CHEO C.C., 1944. — *Chin. J. Sci. Agric.*, 1, 258-263. (D'après *Rev. appl. Mycol.*, 1946, 25, 341.)

COGNEE M., 1963. — *Cot. Fib. trop.*, 18, 249-264.

DJAVADI F., 1969. — *Cot. Fib. trop.*, 24, 175-191.

EVANS G., S. WILHELM et W.C. SNYDER, 1966. — *Phytopath.*, 56, 460-461.

HANSFORD C.G., 1937. — *E. Afr. Agric. J.*, 2, 6, 498-504 ; 3, 1, 79-84. (D'après *Rev. appl. Mycol.*, 1937, 16, 838.)

IN S.Y., 1958. — *Mat. ob. nauch. Sess. Khlopk.*, Tashkent, 2, 230-240. (D'après *Rev. appl. Mycol.*, 1959, 38, 599.)

MASSENOT M., 1964. — *Cot. Fib. trop.*, 19, 383-402.

MILES L.E., 1934. — *Phytopath.*, 24, 558-559.

NADAKAVUKAREN M.J. et C.E. HORNER, 1959. — *Phytopath.*, 49, 527-528 (Abstr.).

RUDOLPH B.A. et G.J. HARRISON, 1944. — *Phytopath.*, 34, 849-860.

SOLOVEVA A.I. et L.V. POLYARKOVA, 1940. — *Tashkent Agric. Publis., Dept. Uzbekistan Soviet Republic.*, 63 pp. (D'après *Rev. appl. Mycol.*, 1948, 27, 19.)

TAUBENHAUS J.J., 1936. — *Texas Agric. Exp. Sta.*, 49th Ann. Rept., 111.

TAUBENHAUS J.J., 1937. — *Proceed Second Ann. Meet. Cott. Dis. Council*, Nashville, Tenn., 8 pp.

WICKENS G.M., 1948. — (D'après *Rev. appl. Mycol.*, 1948, 27, 473.)

CONCLUSION

Our experiments for analyzing the mycoflora with the modified Ulster method have not set forth Verticillium dahliæ *as seed contaminant. Also, the particular methods applied have not permitted to detect the presence of* Verticillium *neither inside nor on the outside of the seeds. Consequently, we think that internal infection of Iranian cotton seeds is improbable.*

CONCLUSIÓN

Nuestras experiencias del análisis de la micoflora por el método de Ulster modificado, no han puesto en evidencia Verticillium dahliæ *como contaminación de las semillas. Igualmente, los métodos particulares empleados no han permitido descubrir la presencia de* Verticillium *ni en el interior ni en el exterior de las simientes. Creemos, por consiguiente, que la infección interna de las semillas iranianas del algodonero es improbable.*

CONTRIBUTION A L'ÉTUDE D'UNE NOUVELLE MALADIE DU COTONNIER : LA VIRESCENCE

I. - Symptômes et importance économique en Haute-Volta en 1968-69

par

R. LAGIÈRE[a], H.D. FRINKING[b] et SOMA OUATTARA[c]

A l'initiative de l'Institut de Recherches du Coton et des Textiles Exotiques et avec l'accord et le soutien du Ministère de l'Agriculture de Haute-Volta, nous avons entrepris une série d'observations et d'études dans le but de mieux connaître cette maladie du cotonnier pour laquelle nous n'avons trouvé que peu de références bibliographiques. Il semble bien, jusqu'à plus ample informé, que ce soit une maladie encore jamais signalée chez le cotonnier, hors de la zone soudanienne malo-voltaïque, où elle sévit de façon endémique depuis plus de vingt ans. On remarque, avec inquiétude, l'intensification de ses manifestations depuis quelques anées.

Les études et observations ont montré d'une façon claire et indubitable qu'il s'agit d'une maladie transmise par greffage et qui n'est causée ni par un champignon ni par une bactérie. Les divers symptômes orientent le diagnostic vers le groupe des « jaunisses » des végétaux ; des études sont en cours sur des micro-organismes « filtrants » colonisant le liber des cotonniers malades (1) et sur des Cicadelles récoltées dans les champs infestés (2).

REVUE BIBLIOGRAPHIQUE

Les « jaunisses » possèdent un ensemble de caractères bien particuliers qui permettaient de les classer à part parmi les maladies dites à virus. Ces singularités ont attiré l'attention des chercheurs et c'est à DOI et col. (1967) que l'on doit les premières observations sur les micro-organismes associés à ces jaunisses. Si l'on n'a pas trouvé de virus proprement dits dans les tissus infectés, on a découvert constamment, par contre, des corps de type mycoplasme dans les cellules libériennes malades (MARAMOROSCH et col. 1968, DOI et col. 1967, ISHIIE et col. 1967, GIANOTTI et col. 1968, MAILLET et col. 1968, COUSIN et col. 1968) et chez les quelques Cicadelles vectrices étudiées (GRANADOS 1964, GIANOTTI et col. 1968, HIRUMI et col. 1969).

Des maladies de type « jaunisse » sont connues chez plus de vingt-cinq plantes principales (BOS 1957) : *Arachis hypogea, Beta vulgaris, Crotalaria juncea, C. usaramoensis, Fraxinus berlandierana, Ipomoea batatas, Lycopersicum esculentum* (= *Solanum lycopersicum*), *Malus communis, Medicago sativa, Sesamum indicum, Solanum tuberosum, S. melongena* pour les plus importants ; *Callistephus chinensis, Carya illinoensis, Juglans* spp., *Justicia gendarussa, Myrica carolinensis, Oryza sativa, Paulownia tomentosa, Prunus persica, Robinia pseudacacia, Rubus idaeus, Saccharum officinale, Syringa amurensis, Tabebuia pallida, Taraxacum koksaghyz, Vaccinium macrocarpon, Vitis vinifera* pour les autres.

Un certain nombre d'hôtes secondaires ont été découverts lors des études de transmission de ces maladies : *Canavalia ensiformis, Capsicum annuum, Convolvulus arvensis, Crotalaria anagyroides, ? C. striata, ? C. verrucosa, Datura stramonium, ? Medicago lupulina, Nicotiana glutinosa, N. rustica, N. tabacum, Solanum demissum, S. nigrum, Vigna sinensis, Vinca rosea* (BOS 1957).

Les *Gossypium* n'ont jamais été mentionnés par des auteurs étrangers. C'est DELATTRE (1965) qui publia le premier une courte note sur cette virescence de la région malo-voltaïque.

L'un ou l'autre des caractères particuliers des jaunisses prévaut en fonction de facteurs peu connus et peut-être suivant le stade de développement de la plante au moment de l'infection (BOS 1964). Quoiqu'il en soit, des maladies des plantes du type « jaunisse » ont été décrites sous les noms de : a) virescence, fausse floraison, pétales verts, *big bud* (SHEAR, 1916, pour l'airelle ; ROBERTSON, 1928, pour le sésame ; DANA 1939, 1940 et HILL, 1943, pour la tomate ; POSNETTE, 1953, pour le fraisier) ; b) phyllodie, avec ou sans prolifération (PAL et NATH, 1935, pour le sésame ; BOSE et MISRA, 1938, pour la crotalaire) ; c) stérilité (ROY, 1931, pour le sésame ; BOJNANSKY et BLATTNY, 1953) ; d) balai-de-sorcière (DOI et col. pour la pomme de terre ; BOS, 1957) ; e) jaunisse (KUNKEL, 1931, pour la reine-marguerite ; COUSIN et MOREAU, 1965) ; f) rabougrissement ou nanisme (SHIKATA et col. 1968, pour le riz ; DOI et col. pour le mûrier ; MARAMOROSCH et col. pour le maïs).

L'importance économique des « jaunisses » est variable selon la plante attaquée. Elles ont causé et

---

a, b : Phytopathologistes à l'I.R.C.T.

c : Ingénieur du Service Voltaïque de la Protection des Végétaux.

(1) Etude par le Pr MAILLET.

(2) Etude par M. J.P. MOREAU, I.N.R.A.

RÉSULTAT

La méthode proposée par NADAKAVUKAREN permet la croissance de nombreux *Aspergillus*, *Fusarium* et *Alternaria* sur le milieu de culture et ne semble pas très spécifique du *Verticillium*. Toutefois, des microsclérotes introduits artificiellement dans l'eau de lavage des semences (environ 10 microsclérotes/ml d'eau de lavage) donnent naissance à des colonies identifiables. De même, la méthode d'EVANS et coll. permet une bonne observation sur les membranes Millipore des microsclérotes, quand ils sont présents.

Nous n'avons pas pu détecter la présence du champignon sur plus de 9 000 semences analysées par les différentes méthodes ; nous sommes amenés à penser que la transmission du *Verticillium dahliae* par les semences est négligeable dans le cas des cotonniers iraniens. Bien entendu, des observations étendues sur plusieurs années seront nécessaires avant d'affirmer entièrement ces résultats.

Nos expérienc...
méthode d'ULSTI...
dence *Verticilliu*...
semences. De m...
ployées n'ont p...
Verticillium ni à...
nes. Nous pens...
interne des sen...
improbable.

ALLEN R.M., 1951 ...
CHEN C.T., S.S. ... *phytopath.* ... *Mycol.*, 1965, ...
CHEO C.C., 1944. — ... *Rev. appl. M...*
COGNEE M., 1963. — *Cot. Fib. trop.*, 18, 249-264.
DJAVADI F., 1969. — *Cot. Fib. trop.*, 24, 175-191.
EVANS G., S. WILHELM et W.C. SNYDER, 1966. — *Phytopath.*, 56, 460-461.
HANSFORD C.G., 1937. — *E. Afr. Agric. J.*, 2, 6, 498-504 ; 3, 1, 79-84. (D'après *Rev. appl. Mycol.*, 1937, 16, 838.)
IN S.Y., 1958. — *Mat. ob. nauch. Sess. Khlopk.*, Tashkent, 2, 230-240. (D'après *Rev. appl. Mycol.*, 1959, 38, 599.)
MASSENOT M., 1964. — *Cot. Fib. trop.*, 19, 383-402.
MILES L.E., 1934. — *Phytopath.*, 24, 558-559.
NADAKAVUKAREN M.J. et C.E. HORNER, 1959. — *Phytopath.*, 49, 527-528 (Abstr.).
RUDOLPH B.A. et G.J. HARRISON, 1944. — *Phytopath.*, 34, 849-860.
SOLOVEVA A.I. et L.V. POLYARKOVA, 1940. — *Tashkent Agric. Publis., Dept. Uzbekistan Soviet Republic.*, 63 pp. (D'après *Rev. appl. Mycol.*, 1948, 27, 19.)
TAUBENHAUS J.J., 1936. — *Texas Agric. Exp. Sta.*, 49th Ann. Rept., 111.
TAUBENHAUS J.J., 1937. — *Proceed Second Ann. Meet. Cott. Dis. Council*, Nashville, Tenn., 8 pp.
WICKENS G.M., 1948. — (D'après *Rev. appl. Mycol.*, 1948,

...coflora with ...t forth Ver... o, the parti... ed to detect ...side nor on ... e think that ... ls is impro...

... a micoflora ... han puesto ... ntaminación ... particulares empleados no han permitido descubrir la presencia de Verticillium *ni en el interior ni en el exterior de las simientes. Creemos, por consiguiente, que la infección interna de las semillas iranianas del algodonero es improbable.*

1969

LAGIÈRE, R., FRINKING, H.D. & OUATTARA, S.

Contribution à l'étude d'une nouvelle maladie du Cotonnier: la virescence. I. Symptômes et importance économique en Haute-Volta en 1968-69. [Contribution to the study of a new disease of Cotton: virescence. I. Symptoms and economic importance in Upper Volta in 1968-69.]

Coton Fibr. trop., 24 (3):317-323. [1 col. pl. (2 fig.), 2 pl. (4 fig.), 2 tables. 31 ref. Engl. Span. summ. I.R.C.T. rue des Renaudes, Paris.]

Microfilm no. 15618

CONTRIBUTION A L'ÉTUDE D'UNE NOUVELLE MALADIE DU COTONNIER : LA VIRESCENCE

I. - Symptômes et importance économique en Haute-Volta en 1968-69

par

R. LAGIÈRE[a], H.D. FRINKING[b] et SOMA OUATTARA[c]

A l'initiative de l'Institut de Recherches du Coton et des Textiles Exotiques et avec l'accord et le soutien du Ministère de l'Agriculture de Haute-Volta, nous avons entrepris une série d'observations et d'études dans le but de mieux connaître cette maladie du cotonnier pour laquelle nous n'avons trouvé que peu de références bibliographiques. Il semble bien, jusqu'à plus ample informé, que ce soit une maladie encore jamais signalée chez le cotonnier, hors de la zone soudanienne malo-voltaïque, où elle sévit de façon endémique depuis plus de vingt ans. On remarque, avec inquiétude, l'intensification de ses manifestations depuis quelques anées.

Les études et observations ont montré d'une façon claire et indubitable qu'il s'agit d'une maladie transmise par greffage et qui n'est causée ni par un champignon ni par une bactérie. Les divers symptômes orientent le diagnostic vers le groupe des « jaunisses » des végétaux ; des études sont en cours sur des micro-organismes « filtrants » colonisant le liber des cotonniers malades (1) et sur des Cicadelles récoltées dans les champs infestés (2).

REVUE BIBLIOGRAPHIQUE

Les « jaunisses » possèdent un ensemble de caractères bien particuliers qui permettaient de les classer à part parmi les maladies dites à virus. Ces singularités ont attiré l'attention des chercheurs et c'est à DOI et col. (1967) que l'on doit les premières observations sur les micro-organismes associés à ces jaunisses. Si l'on n'a pas trouvé de virus proprement dits dans les tissus infectés, on a découvert constamment, par contre, des corps de type mycoplasme dans les cellules libériennes malades (MARAMOROSCH et col. 1968, DOI et col. 1967, ISHIIE et col. 1967, GIANOTTI et col. 1968, MAILLET et col. 1968, COUSIN et col. 1968) et chez les quelques Cicadelles vectrices étudiées (GRANADOS 1964, GIANOTTI et col. 1968, HIRUMI et col. 1969).

Des maladies de type « jaunisse » sont connues chez plus de vingt-cinq plantes principales (BOS 1957) : *Arachis hypogea, Beta vulgaris, Crotalaria juncea, C. usaramoensis, Fraxinus berlandierana, Ipomoea batatas, Lycopersicum esculentum* (= *Solanum lycopersicum*), *Malus communis, Medicago sativa, Sesamum indicum, Solanum tuberosum, S. melongena* pour les plus importants ; *Callistephus chinensis, Carya illinoensis, Juglans* spp., *Justicia gendarussa, Myrica carolinensis, Oryza sativa, Paulownia tomentosa, Prunus persica, Robinia pseudacacia, Rubus idaeus, Saccharum officinale, Syringa amurensis, Tabebuia pallida, Taraxacum koksaghyz, Vaccinium macrocarpon, Vitis vinifera* pour les autres.

Un certain nombre d'hôtes secondaires ont été découverts lors des études de transmission de ces maladies : *Canavalia ensiformis, Capsicum annuum, Convolvulus arvensis, Crotalaria anagyroides, ? C. striata, ? C. verrucosa, Datura stramonium, ? Medicago lupulina, Nicotiana glutinosa, N. rustica, N. tabacum, Solanum demissum, S. nigrum, Vigna sinensis, Vinca rosea* (BOS 1957).

Les *Gossypium* n'ont jamais été mentionnés par des auteurs étrangers. C'est DELATTRE (1965) qui publia le premier une courte note sur cette virescence de la région malo-voltaïque.

L'un ou l'autre des caractères particuliers des jaunisses prévaut en fonction de facteurs peu connus et peut-être suivant le stade de développement de la plante au moment de l'infection (BOS 1964). Quoiqu'il en soit, des maladies des plantes du type « jaunisse » ont été décrites sous les noms de : a) virescence, fausse floraison, pétales verts, *big bud* (SHEAR, 1916, pour l'airelle ; ROBERTSON, 1928, pour le sésame ; DANA 1939, 1940 et HILL, 1943, pour la tomate ; POSNETTE, 1953, pour le fraisier) ; b) phyllodie, avec ou sans prolifération (PAL et NATH, 1935, pour le sésame ; BOSE et MISRA, 1938, pour la crotalaire) ; c) stérilité (ROY, 1931, pour le sésame ; BOJNANSKY et BLATTNY, 1953) ; d) balai-de-sorcière (DOI et col. pour la pomme de terre ; BOS, 1957) ; e) jaunisse (KUNKEL, 1931, pour la reine-marguerite ; COUSIN et MOREAU, 1965) ; f) rabougrissement ou nanisme (SHIKATA et col. 1968, pour le riz ; DOI et col. pour le mûrier ; MARAMOROSCH et col. pour le maïs).

L'importance économique des « jaunisses » est variable selon la plante attaquée. Elles ont causé et

---

a, b : Phytopathologistes à l'I.R.C.T.

c : Ingénieur du Service Voltaïque de la Protection des Végétaux.

(1) Etude par le Pr MAILLET.

(2) Etude par M. J.P. MOREAU, I.N.R.A.

causent encore, pour certaines, des pertes économiques sérieuses chez les plantes cultivées (MARAMOROSCH et col., 1968). Des plantes introduites dans de nouvelles régions de culture ont été quelquefois attaquées par des « jaunisses » inconnues antérieurement dans ce milieu et qui ont été découvertes plus tard sur des plantes locales chez lesquelles elles ne provoquaient que peu ou pas de dommages. Ce fut le cas pour *Beta vulgaris* et *Prunus persica* (BENNETT, 1967). Les maladies les plus destructrices causées par les micro-organismes transmis par les Cicadelles apparaissent dans les zones agricoles nouvelles du monde à la suite, donc, de l'introduction de plantes cultivées et de la plus large dissémination des insectes vecteurs. En effet, l'agent causal se conserve et se multiplie chez plusieurs hôtes végétaux mais aussi, pour certains, chez les Cicadelles vectrices (MARAMOROSCH, 1952).

Nous estimions l'an dernier (LAGIERE, 1968) que la Virescence du cotonnier prenait une allure épidémique dans l'ouest de la Haute-Volta, après être restée une vingtaine d'années à l'état endémique. Cette extension rapide était mise en parallèle avec l'amélioration de la technique culturale. Les pertes étaient rapidement estimées à 1 % pour l'ensemble du pays, soit quelque six millions de francs C.F.A. de manque à gagner pour les cultivateurs. Cette année (1968-1969), la maladie a continué à progresser et l'on a noté des relations étroites entre l'importance de la Virescence, d'une part, et la tardiveté de la date de semis (relation négative) ou la luxuriance de la végétation (relation positive) d'autre part.

RÉSULTATS

Les observations ont été effectuées dans toute la zone cotonnière voltaïque mais plus particulièrement dans l'ouest et plus précisément à la Ferme de multiplication de la C.F.D.T. à BONI. Nous présentons dans cette note nos observations, d'une part sur les symptômes et l'évolution de la maladie et, d'autre part, sur l'importance économique de cette Virescence.

a) Symptômes et évolution de la maladie

Les premiers symptômes sont notés avec quelque certitude à partir du 45-50e jour de végétation des cotonniers, mais certains étaient apparents bien avant dans quelques cas de rabougrissement et de jaunissement du feuillage. La maladie peut donc provoquer soit le rachitisme et le jaunissement généralisé du feuillage accompagné d'anomalies des pièces florales, soit, le plus souvent, des transformations florales (régression florale ou antholyse, selon les auteurs) sans qu'il y ait, encore, des perturbations visibles dans la croissance.

Les *jeunes boutons floraux* ont un aspect anormal quelques jours après leur individualisation nette : les bractées s'écartent et dégagent le bouton floral qui a la grosseur d'un pois, à l'exemple de ce que l'on observe après des morsures d'*Earias* (fig. 1).

Le *calice* gamosépale est atteint de gigantisme (fig. 2) ; il se colore de rouge-violacé au sommet. Les jeunes pétales, encore repliés, apparaissent au fond de ce calice géant, non plus de couleur jaune très pâle, mais virant progressivement au vert et se teintant de rouge-violacé.

Les *bractées* et le pédoncule peuvent être atteints de gigantisme (fig. 3 et 4), le pédoncule mesurant parfois 10 cm et plus.

La *corolle* s'épanouit prématurément et les pétales verts débordent à peine du calice. La disposition hélicoïdale des pétales reste toujours très apparente même après qu'ils aient atteint une taille plus normale. La corolle est beaucoup plus aplatie que chez les fleurs saines et les pétales verts acquièrent plus ou moins rapidement une structure épaisse, foliacée ; ils deviennent persistants comme tout organe de l'appareil végétatif. Ils se colorent plus ou moins intensément en rouge.

L'*ovaire* est apparemment normal dans les premiers temps, mais le style et les étamines sont souvent modifiés et d'aspect très variable : allongés, atrophiés, en masse ou absents, coloration verte teintée de rouge. Les pièces florales se transforment en organes végétatifs persistants, solidement soudés entre eux. L'ovaire grossit un peu, fait éclater la gaine staminale. Les ovules sont atteints d'hypertrophie, font éclater l'ovaire et prolifèrent quelquefois à l'extérieur en structures foliacées.

La *fleur virescente* est largement ouverte, de couleur verte avec de fortes imprégnations rouge-violacé ; toutes ses pièces sont transformées et elle acquiert avec son pédoncule érigé un comportement d'organe végétatif persistant.

Dans la quinzaine qui suit la fin de l'incubation, des *bourgeons axillaires* des nœuds inférieurs, médians puis supérieurs se développent et donnent naissance à des branchettes grêles, érigées, portant de petites feuilles jaunâtres et des organes floraux virescents. Ces croissances secondaires, qui ne constituent pas de balais-de-sorcières, accompagnent un ralentissement dans la multiplication des méristèmes terminaux des sympodes et des monopodes ; les entre-nœuds sont courts ; les feuilles de taille réduite, jaunâtres, aux pétioles et nervures principales plus ou moins rougeâtres, au limbe bordé puis coloré de rouge-lilas constituent avec les fleurs virescentes des bouquets terminaux caractéristiques.

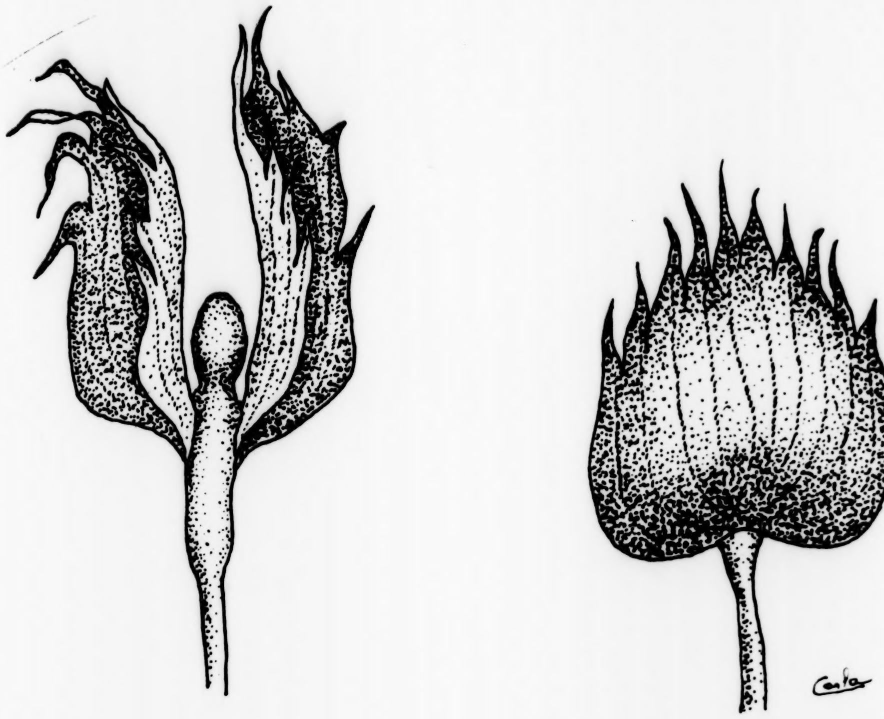
Trois semaines après le début de la maladie, les cotonniers sont atteints très visiblement de *jaunisse;* l'ensemble du feuillage de la moitié supérieure des plants présente les caractères ci-dessous : le limbe est plus ou moins gaufré et frappé d'épinastie ; toutes les croissances nouvelles sont dressées ; le feuillage de la moitié inférieure est de couleur vert-jaunâtre avec des infiltrations diffuses de rouge. Les organes fructifères vert-rougeâtre sont tous présents.

La Virescence peut frapper les plants en début, au milieu ou en fin de fructification. Tous les boutons floraux formés après les premières manifestations seront anormaux et stériles. Ceux au dévelopement légèrement plus avancé à ce moment-là peuvent aussi évoluer anormalement dans les dix-quinze jours qui

PLANCHE I

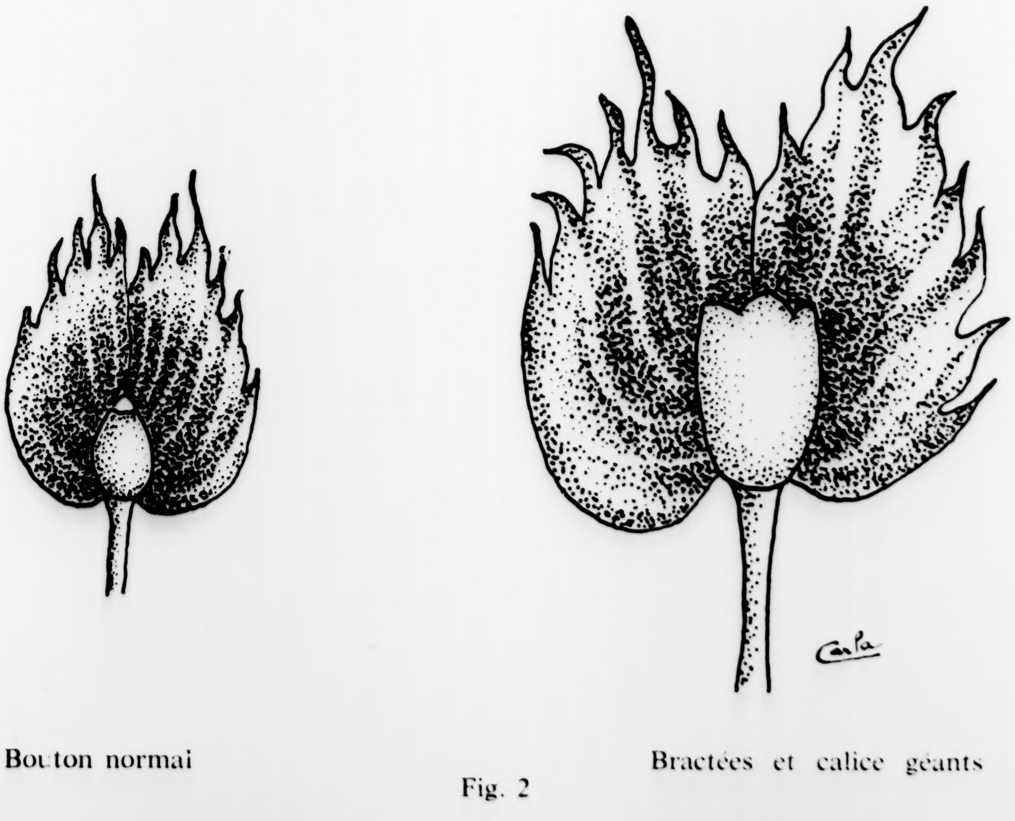
Symptômes de virescence (phyllodie) chez le cotonnier *G. hirsutum* en Haute-Volta : calicule, calice, géants ; pétales foliacés ; coloration rougeâtre caractéristique (Photos de R. DELATTRE, 1967)

PLANCHE II



Bouton virescent | Bouton normal

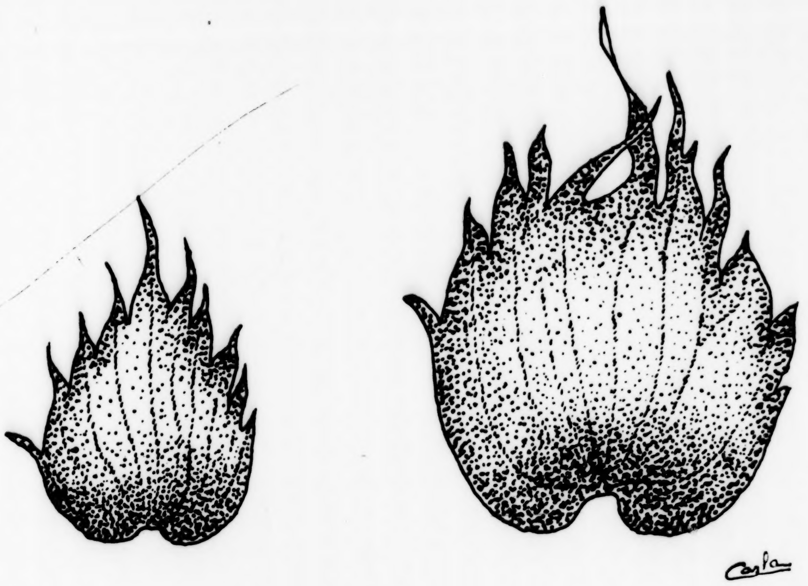
Fig. 1



Bouton normal | Bractées et calice géants

Fig. 2

PLANCHE III



Bractée normale

Fig. 3

Bractée géante

Fig. 5
Un cas de prolifération

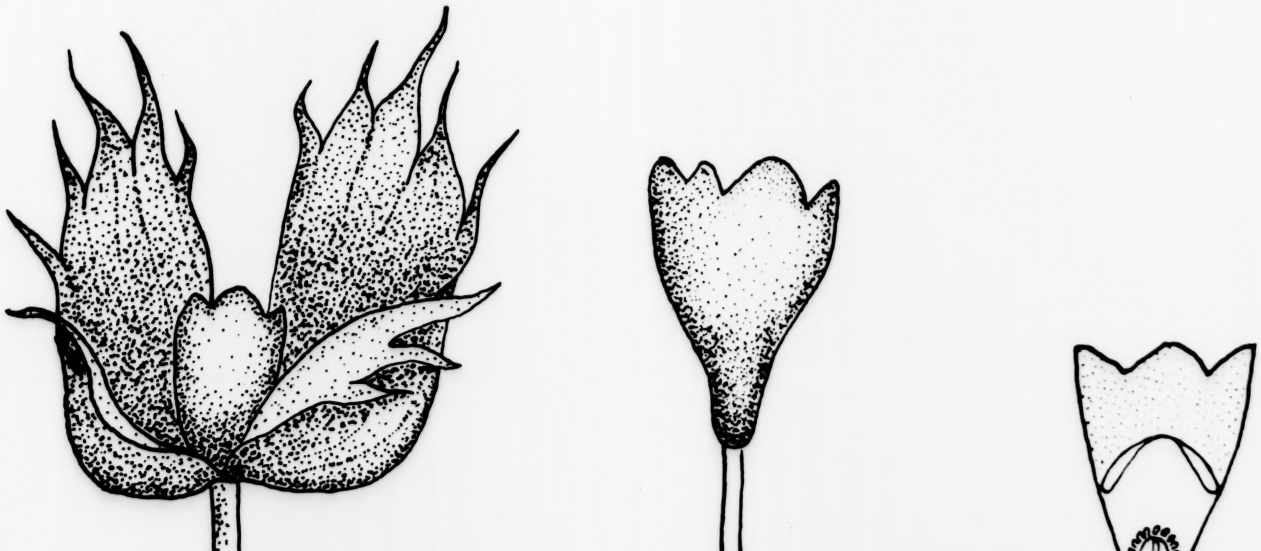


Fig. 4
Calice et pédoncules géants

suivent. On peut donc observer les premiers symptômes floraux chez :

a) un cotonnier de port normal

— le premier bouton (I-1) est atteint, tous les autres le seront ; l'évolution sera celle décrite plus haut ;

— la maladie apparaît en premier lieu chez un bouton formé après le début de la floraison ;

— les productions ultérieures seront virescentes ;

— les fleurs ou jeunes capsules antérieures pourront également être modifiées ; par exemple :

- la corolle s'épanouit normalement mais un secteur présente de légères anomalies classiques ; le style deviendra végétatif et persistera sur l'ovaire ;
- la jeune capsule arrête très tôt sa croissance.

b) un cotonnier déjà marqué de jaunisse

— tous les boutons sont virescents.

b) Importance économique de la virescence du cotonnier

Des évaluations et des sondages ont été effectués sur le territoire de l'Office Régional de Développement (O.R.D.) de la Volta-Noire, du Secteur de BOBO-DIOULASSO et de l'O.R.D. de OUAGADOUGOU.

1 - *Dans l'O.R.D. de la Volta-Noire*

Plus de 50 % de la production cotonnière voltaïque sont récoltés sur le territoire de l'O.R.D. de la Volta-Noire.

Les semis ont souvent été effectués très tôt, fin mai début juin, et les premières manifestations de la Virescence ont été signalées dès le début du mois de juillet dans certains champs, mi-juillet, fin juillet et début août dans d'autres. Un paysan (M. Brahima SAWADOGO, du village Kéra) affirme avoir remarqué un cotonnier atteint de jaunisse et rabougri dès la fin du mois de juin.

Les premières manifestations frappantes sont signalées 50 à 60 jours après le semis mais certains chefs de Sous-Secteur et certains cultivateurs dignes de confiance en ont remarqué 45 et 40 jours après le semis (villages Dankuy, Dionkongo, Safané, Sokoula et Samakuy).

A la fin du mois de septembre, les enquêtes conduites par l'équipe de recherche et les comptages effectués par les chefs de Sous-Secteur, les chefs de Centre et les Moniteurs ont donné les chiffres rapportés dans le tableau 1.

Les quelque 40 points d'observation ci-dessus donnent une idée de l'importance de la Virescence à la mi-septembre. Les plants repérés ont tous été attaqués en début ou au milieu de la période de fructification et leur potentiel de production est donc totalement ou largement annulé. Les 15 observations du Secteur de DÉDOUGOU aboutissent à une moyenne de 2,7 % de plants atteints au 15 septembre, avec des extrêmes de 7,3 % à Safané et 0,3 % à Tiékuy. On obtient à peu près les mêmes résultats dans le Secteur de Houndé avec 2,9 % de moyenne et des extrêmes de 14,6 % (Boni) et 0,3 % (Koumbia).

Les Secteurs de DÉDOUGOU et de HOUNDÉ sont particulièrement infestés de Virescence mais les autres ne sont pas indemnes ; ainsi, de nombreux cas ont été signalés dans les Secteurs de Solenzo et de Nouna ; il serait bien étonnant que ceux de Kougny, Sourou et Tougan soient épargnés bien que nous ne possédions aucune information à leur sujet.

2 - *Dans le Secteur de* BOBO-DIOULASSO

A la fin du mois d'août, cinq centres sur sept étaient sérieusement infestés. Les deux encore indemnes — N'DOROLA et SINDOU — avaient été ensemencés plus tardivement que les cinq autres et cela peut expliquer un décalage des premières manifestations.

Les premiers symptômes de Virescence ont été signalés vers le 10-15 août dans les plantations des villages de Lahirasso, Kourouma et Koloko. Les semis avaient été effectués dans la dernière décade du mois de mai ; les anomalies ont été relevées 50 à 55 jours après le semis, au moment des premières fleurs.

A la fin du mois d'août, donc 45-50 jours après les premières manifestations, une enquête conduite par les Moniteurs de la C.F.D.T. donnait les renseignements figurant au tableau 2.

A la fin du mois d'août, les 57 champs visités contenaient, en moyenne, 1 % de cotonniers virescents, soit 1 % de plants totalement stérilisés. Il est probables que les observations de la fin septembre et celles de la fin octobre fassent apparaître un progrès rapide de la maladie dans ces champs et dans ceux non encore visités.

Une tache de 50 m² portant une quinzaine de cotonniers atteints de Virescence était découverte le 11 septembre au Centre Agricole Polyvalent de Matourkou.

3 - *Dans l'O.R.D. de* OUAGADOUGOU

Le Directeur de l'O.R.D. de OUAGADOUGOU nous informe par lettre des résultats au 31 octobre des sondages effectués.

« A la suite de l'enquête menée par les chefs de Secteurs sur la Phyllodie du cotonnier, il apparaît que cette maladie n'a qu'une faible incidence dans l'O.R.D. de OUAGADOUGOU. Les comptages de plants atteints permettent de distinguer deux régions suivant l'importance de l'attaque.

a - Une première région à peu près indemne comprend le sud de Boussé et de Ziniaré, Ouagadougou, Kombissiri, Saponé, Pô et Tiébélé.

b - Le reste de l'O.R.D a subi des attaques moyennes à faibles. »

Tableau 1. — *Résultats des prospections sur l'importance relative de la Virescence du cotonnier dans les plantations jusqu'au mois de septembre* (1)

Secteur	Sous-Secteur	Centre	Date de semis	Epoque repérage 1er symptôme	Nombre plants virescents — Nombre	%
Secteur de DEDOUGOU						
	S/s Dédougou					
		Centre Dédougou	30/5	24/7	838	3,4
		Souri	29/5	22/7	1 249	4,9
		Kari	1/6	8/8	716	2,0
	S/s Ouarkoye					
		Centre Poundou	15/5	4/7	260	1,0
		Miana	15/6	5/8	300	1,2
		Diekuy	25/5	30/7	235	1,3
	S/s Safané					
		Centre Kinséré	28/5	26/7	134	0,5
		Ouana	21/5	21/7	855	3,4
					625	2,5
		Nana	2/6	30/7	260	1,0
		Safané	1/6	1/8	1 836	7,3
					813	3,2
					608	2,4
		Tiékuy	1/6	3/8	87	0,3
	S/s Thériba					
		Centre Labien	29/5	25/7	990	3,9
		Tierkou	25/5	30/7	84	0,3
		Oula	5/6	10/8	184	0,7
Moyenne						2,7
Secteur de HOUNDE						
	S/s Boromo					
		Centre Boromo	10/6	29/7	452	1,4
		Oury	25/6	28/7	522	1,6
	S/s Boni					
		Centre Boni	18/6	18/7	4 180	14,6
		Kopoye	6/6	20/7	1 220	4,0
	S/s Koumbia					
		Centre Koumbia	25/7	9/9	130	0,3
		Kari	25/6	10/8	258	0,6
		Pê	19/6	6/8	2 058	7,2
	S/s Béréba					
		Centre Sara	10/6	10/8	149	0,6
		Béréba	5/6	1/8	559	2,1
		Bokuy	8/6	1/8	637	2,0
		Popioho	22/5	6/7	1 349	5,0
		Siéni	5/6	29/7	838	3,0
		Kiéré	4/6	3/8	347	1,1
	S/s Bagassi					
		Centre Mouni	24/6	24/7	395	1,2
		Mamon	15/6	15/7	500	1,5
		Pompoy	15/5	28/7	2 700	9,1
		Mana	24/5	9/7	750	3,1
		Komkoliko	25/5	18/7	680	2,1
		Vy	10/6	20/7	190	0,7
		Pahin	30/6	13/7	360	1,4
		Bandio	8/6	27/7	470	1,5
		Bagassi	17/6	16/7	236	0,8
		Koho	12/6	14/7	175	0,5
		Yaramoko	27/5	21/7	940	3,7
			—	24/7	1 126	4,1
	S/s Houndé					
		Centre de Bouéré	2/6	19/7	715	3,7
Moyenne						2,9

(1) Tous les champs ne sont pas examinés ; l'échantillon ci-dessus n'est peut-être pas absolument représentatif.

Tableau 2. — *Résultats des premiers sondages, à la fin du mois d'août, dans le secteur de* BOBO-DIOULASSO *: villages et plantations dans lesquels ont été découverts des cas de Virescence*

Centre et Village	Date de semis	Epoque repérage 1er symptôme	Nombre plants virescents Nombre	%
Centre de Lahirasso				
Vil. Bossora	28/5	20-22/8	8 800	1,0
Vil. Lahirasso	juin	21-26/8	10 500	0,9
Vil. Bakornisso	fin mai	26/8	5 500	1,3
Centre de Kourouma				
Vil. Kourouma (1)	7-15/6	20-26/8	5 500	2,7
Centre de Somorogouan				
Vil. Silorla	25/6	28/8	11	—
Vil. Fananga	18/6	28/8	14	—
Centre de Koloko				
Vil. Sintasso	1/6	15/8	155	1,3
Vil. Kartasso	1-26/6	16/8	210	0,3
Vil. Mahon	26/6	20/8	18	—
Vil. Natindougou	20/6	7/8	60	0,4
Centre de Sifarasso				
Vil. Sifarasso	5/6	2/8	26	—
Vil. Sokoroni	30/6	27/8	14	—
Vil. Outila	4/6	19/8	35	0,5
Total et moyenne			30 923	1,0

(1) Plantation de M. Traoré GNABERE : 1,50 ha, 3 800 cotonniers malades = 5 %.

Il semble donc, en définitive, qu'un certain pied-de-cuve existe dans le territoire de l'O.R.D. de Ouagadougou mais que des conditions particulières sont défavorables à l'extension de la maladie. L'amélioration des techniques culturales et l'intensification de la production ne modifieront-elles pas le milieu dans un sens favorable à la virescence ?

DISCUSSION

Les symptômes des jaunisses ont été bien étudiés par divers auteurs dont MARAMOROSCH qui les a résumés ainsi en 1968 : stimulation vers la croissance de bourgeons normalement dormants ; développement de rejets axillaires grêles ; éclaircissement des nervures ; chlorose sans tache ; virescence des fleurs ; malformations ; rabougrissement ; croissance érigée anormale. Les observations de BOS (1957) précisent certains aspects : la chloranthèse (virescence) est suivie de phyllodie (pièces florales acquerrant une structure foliacée), d'apostasis (allongement de l'entre-nœud sous le calice) et de prolifération (développement de rameaux feuillus à la place des pièces florales). Finalement, la fleur est remplacée par une branche feuillue.

ROBERTSON (1928) chez *Sesamum indicum* puis BOSE et MISRA (1938) chez *Crotalaria juncea* notent les premières anomalies à partir du début de la floraison ; les organes floraux sont transformés en structures vertes foliacées tandis qu'apparaissent de très nombreux rameaux.

Les symptômes observés en 1968 chez le cotonnier dépassent ceux d'une simple virescence. Le stade « phyllodie », au sens de BOS, a été atteint. Après les premières manifestations de la maladie — tératologiques chez le calice — l'antholyse se poursuit par la virescence de la corolle suivie ou précédée d'altérations anatomiques profondes conduisant à une structure foliacée, épaisse, des sépales et des pétales. Cette évolution vers la transformation en feuilles des pièces florales est le plus souvent limitée aux sépales, pétales et ovules ; nous avons observé, en outre, quelques cas dans lesquels la totalité des pièces florales étaient remplacées par une lame foliacée. Tout cela, ajouté à la prolifération de certaines parties de la fleurs (fig. 5) et à l'hypertrophie du calice nous rapproche beaucoup de ce que l'on sait des Phyllodies.

Tout permet de supposer que le cotonnier peut être infecté naturellement dès son plus jeune âge. La maladie peut ensuite se manifester à la fois sur l'appareil végétatif et sur les organes de reproduction mais, le plus souvent, elle se limite dans les premières phases aux seuls boutons floraux.

La durée de l'incubation chez le cotonnier après l'inoculation naturelle par vecteur est encore inconnue : elle pourrait être de 20 à 30 jours. Le premier bouton floral est bien individualisé 45 jours après la germination et il peut déjà présenter des symptômes de la Virescence, dont l'écartement des bractées (fig. 1), puis le gigantisme du calice (fig. 2). L'inoculation se serait donc placée dans une période allant du 15e au 25e jour après le semis.

Le raccourcissement des entre-nœuds, l'émission de branchettes grêles à partir de boutons axillaires, la production de petites feuilles jaunâtres, plus ou moins teintées de rouge, le géotropisme négatif des nouvelles croissances dont certaines sont groupées en bouquets terminaux, puis le jaunissement progressif du plant entier sont les symptômes complémentaires qui précisent bien que nous sommes en présence d'une maladie appartenant au groupe des « jaunisses ». Ce groupe qui doit son nom à l'aspect final des plants inclut des Virescences, des Phyllodies et des « Balais-de-Sorcières ». Le cas du cotonnier constitue un des premiers exemples, sinon le premier, d'étude de « jaunisse africaine ». Les manifestations de Haute-Volta sont les premières — et peut-être les seules — de jaunisse du cotonnier. On peut espérer de nombreux points communs avec les jaunisses américaines, européennes et asiatiques qui s'attaquent à d'autres plantes que le cotonnier.

On sait que certaines jaunisses sont associées à des micro-organismes filtrants apparentés à des mycoplasmes. Les échantillons de cotonnier virescent adressés à Mme M.-T. COUSIN (I.N.R.A.) et étudiés par le Pr MAILLET (Faculté des Sciences, Rennes) contiennent eux-mêmes des corps analogues à des mycoplasmes. Ces auteurs présenteront une communication.

La virescence du cotonnier présente un caractère certain de gravité dans les régions de l'ouest où la culture cotonnière s'améliore rapidement : O.R.D. de la Volta-Noire et Secteur de Bobo-Dioulasso. Bien rares sont les villages indemnes de cette maladie.

Des comptages précis effectués dans des essais comparatifs situés toujours sur le même terrain montrent que l'incidence économique de la maladie a doublé et parfois triplé depuis l'an dernier :

	% de cotonniers virescents	
Kari (Houndé)	1967	1968
— Essai variétal	4,1	9,0
— Essai « soustractif »	1,3	2,7
— Essai de fumure	1,4	4,7
	2,3	5,5
Boni		
— Essai « soustractif »	—	2,7
— Essai de fumure	—	3,8

Les dénombrements de cotonniers atteints dans les champs sont aussi éloquents : au 15 septembre, 2,7 % dans le Secteur de Dédougou, 2,9 % dans le Secteur de Houndé ; au 31 août, 1 % dans le Secteur de Bobo-Dioulasso.

Ces chiffres sont des moyennes qui cachent parfois des situations graves (10 à 15 %) ou catastrophiques (80 %), celles-ci n'étant pas retenues pour le calcul des moyennes. Les comptages n'ont recensé que des cotonniers atteints en début de fructification, c'est-à-dire qui seront totalement stérilisés. Les dommages représenteront cette année de 2 à 3 % du potentiel de production de l'ouest de la Haute-Volta. La production cotonnière progresse rapidement dans cette région : 4 000 t en 1965-66, 8 500 t en 1966-67, 10 500 t en 1967-68 et 19 000 t en 1968-69. Cette situation, apparemment très florissante, masque les manques à gagner imputables à la Virescence et pourrait les faire apparaître comme négligeables aux yeux des autorités responsables. En réalité, les 2 à 3 % de plants stériles mais très végétatifs s'accompagnent bien d'un manque à récolter de 2 à 3 %, soit 400 à 600 t de coton-graine en 1968-69, soit encore 12 à 15 millions C.F.A. en moins pour les cultivateurs de cette région. Le problème mérite la plus grande attention.

REMERCIEMENTS

Les auteurs remercient vivement M. SIRY, Directeur du Développement Rural au Ministère de l'Agriculture, les directions générales et locales de la Compagnie Française pour le Développement des Textiles (C.F.D.T.) et de l'Institut de Recherches d'Agronomie Tropicale (I.R.A.T.) pour toute l'aide qu'ils en ont reçue. Ils remercient également Mme COUSIN et le Pr MAILLET pour les études auxquelles ils se sont livrés sur des échantillons de cotonniers virescents.

RÉSUMÉ

Une maladie du type « jaunisse » est décrite chez le cotonnier. Les symptômes ne s'écartent pas de ceux connus pour ce genre d'infection : virescence et phyllodie sans balai-de-sorcière. L'ouest de la Haute-Volta est principalement infesté ; les pertes atteignent 2,7 % de la production, en moyenne.

BIBLIOGRAPHIE

BENNET C.W., 1967. — Epidemiology of leafhopper - transmitted viruses. *Ann. Rev. Phytopath.*, 5, 87-108

BOSNANSKY V. et C. BLATTNY, 1953. — Virozne Zltenky a bezsemennostive svetle sovietskych a nasich vyskumov. *Biologia*, 8, 538-560.

BOS L., 1957. — Heksenbezemverschijnselen. Een Pathologisch - Morfologisch onderzoek. Lab. voor Plantensystematick en-geografic. Land bouwhogeschool, Wagennigen. *Ed. Veenman & Zonen, Wageningen.*

BOS L., 1964. — Symptoms of virus diseases in plants - Inst. for Phytopath. Res., Wageningen. *Centre for Agric. Public. and Docum.*, 2e *Ed.*

BOSE R.D. et S.D. MISRA, 1938. — Studies in Indian fibre plants. n° 6 : Phyllody and some other abnormalities in the flower of sunn hemp (*Crotalaria juncea* L.). *Ind. J. Afric. Sci.*, 8, 4, 417-423.

CHIYKOWSKI L.N., 1952. — Clover phyllody in Canada and its transmission. *Canad. J. Bot.*, 40, 397-403.

COUSIN M.T., P.L. MAILLET J.P. GOURRET, C. GRISON et T. STARON, 1968. — Présence de particules de type mycoplasme dans le phloème de plantes infectées par trois maladies de type « jaunisse européenne » : le stolbur de la tomate, l'Aster Yellows du glaïeul, la phyllodie du trèfle. Etudes cytologiques et ultrastructurales. Premiers essais de lutte chimique. *C.R. Acad. Agric. France*, n° 11, 9 et 16 oct., 887-893.

COUSIN M.T. et J.P. MOREAU, 1965. — Les maladies à virus de type « jaunisse européenne ». *C.R. Journées Phyt. Phytoph. Circummédit.*, Marseille, sept.

DANA B.F., 1939. — Morphological and anatomical features of Phyllody in varieties of tomato and bean. *Phytopath.*, 29, 823 (abstr.).

DANA B.F., 1940 — The « big bud » of tomato in the Pacific N.W. *Phytopath.*, 30, 866-869.

DELATTRE R., 1965. — La Virescence du cotonnier. I Recherches préliminaires. *Cot. Fib trop.*, 20, 2, 289 294.

DELATTRE R., 1968. — La Virescence du cotonnier ; deuxième note. *Cot. Fib. trop.*, 23, 3, 386-390 (comm brève).

DOI Y., M. TERENAKA, K. YORA et H. ASUYAMA, 1967. — Mycoplasma - or PLT group like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches-broom, aster yellows or paulownia witches-broom. *Ann. Phytopath. Soc. Japan*, 33, 259-266 (som. anglais).

GIANOTTI J., G. DEVAUCHELLE et C. VAGO, 1968. — Micro-organismes de type mycoplasme chez une Cicadelle et une plante infectée par la phyllodie. *C.R. Acad. Sci., Paris*, 266, 2168-2170.

GIANOTTI J., G. MORVAN et C. VAGO, 1968. — Micro-organismes de type mycoplasme dans les cellules libériennes de *Mallus sylvestris* L. atteint de la maladie des proliférations. *C.R. Acad. Sci., Paris*, 267, 76-77.

GIANOTTI J., G. MARCHOUX et C. VAGO, 1968. — Micro-organismes de type mycoplasme dans les celulles libériennes de *Solanum lycopersicum* L. atteint de Stolbur. *C.R. Acad. Sci., Paris*, 267, 454-456.

GRANADOS R.R., 1964. — Cité par MARAMOROSCH et Coll., 1968.

HILL A.V., 1943. — Insect transmission and host plants of virescence (big bud of tomato). *J. Counc. Sci. Industr. Res.*, 16, 85-90.

HIRUMI H. et K. MARAMOROSCH, 1969. — Mycoplasma-like agents of aster yellows in the salivary gland of leafhopper vectors. *Phytopath.*, 59, 4, 399 (abstr.).

ISHIIE T., Y. DOI, K. YORA et H. ASUYAMA, 1967. — Suppressive effects of antibiotics of tetracycline group on symptom development of mulbury dwarf disease. *Ann. Phytopath. Soc. Japan*, 33, 267-275 (som. anglais).

KUNKEL L.O., 1931. — Studies on Aster yellows. *Contr. Boyce Thompson Inst., Plt Res.*, 3, 85-123.

LAGIERE R., 1968. — Enquête sur l'importance de la Virescence du cotonnier en Haute-Volta. *Cot. Fib. trop.*, 23, 2, 279-286 (note technique).

LAGIERE R., H. FRINKING et Soma OUATTARA, 1969. — Etudes et observations préliminaires sur la Virescence du cotonnier en Haute-Volta. Rapport ronéotypé, mars 1969.

MAILLET P.L., J.P. GOURRET et C. HAMON, 1968. — Sur la présence de particules de type mycoplasme dans le liber de plantes atteintes de maladies du type « jaunisse » (Aster yellows, Phyllodie du trèfle, stolbur de la tomate) et sur la parenté ultra-structurale de ces particules avec celles trouvées chez divers Homoptères. *C.R. Acad. Sci., Paris*, 266, 2309-2311.

MARAMOROSCH K., 1952. — Direct evidence for the multiplication of aster yellows virus in its insect vector. *Phytopath.*, 42, 49-64.

MARAMOROSCH K., Eishiro SHIKATA et Robert R. GRANADOS, 1968. — Structures ressmbling mycoplasma in diseased plants and in insect vectors. *Trans. N.Y. Acad. Sci.* (*Ser.* 2), 30, 6, 841-855.

PAL B.P. et Puskkar NATH, 1935. — Phyllody : a possible virus disease of *Sesamum*. *Ind. J. Agric. Sci.*, 5, 4, 517-522.

POSNETTE A.F., 1953. — Green petal, a new virus disease of strawberries. *Plt Pathol.*, 2, 17-18

ROBERTSON H.F., 1928. — Annual report of the Mycologist Burma, for the year ended 30th june 1928. *Rev. appl. Mycol.*, 8, 355 (abstr.).

ROY S.C., 1931. — A preliminary note on the occurence of sepaloidy and sterility in til (*Sesamum indicum*). *Agric. Livest. India*, 1, 282-285.

SHEAR C.L., 1916. — False blossom of the cultivated cranberry. *U.S.D.A. Bull.*, 444.

SUMMARY

A disease of the « yellows » type in cotton is described. The symptoms are not very much different from those known for that sort of infection : virescence and phyllody without witches brooms. The infestation occurs chiefly in the West of Upper Volta. Average production losses amount to 2,7 %.

RESUMEN

Una enfermedad del tipo « ictericia » se describe en el algodonero. Los sintomas no se apartan de aquellos conocidos para esta clase de infección : virescencia y filodia sin escoba-de-bruja. El oeste del Alto Volta se encuentra principalmente infestado ; las pérdidas alcanzan 2,70 % de la producción, como término medio.

1969

GOURRET, J.P. & MAILLET, P.L.

Ultrastructure des mycoplasmes dans le phloème du Cotonnier atteint de virescence. [Ultrastructure of mycoplasms in the phloem of Cotton affected by virescence.]

<u>Coton Fibr. trop</u>., <u>24</u> (3):325-326. [3 Pl. (5 fig.). 22 ref. Engl. Span. summ. Fac. Sci. Rennes]

<u>Microfilm no</u>. 15619

ULTRASTRUCTURE DES MYCOPLASMES DANS LE PHLOÈME DU COTONNIER ATTEINT DE VIRESCENCE

par

J. P. GOURRET et P. L. MAILLET

Laboratoire de Biologie Cellulaire, ERA 129
Faculté des Sciences de Rennes.

La virescence du cotonnier (*Gossypium hirsutum L.*) est connue depuis plus de vingt ans en Haute-Volta. Elle fut décrite pour la première fois en 1965 (DELATTRE, 1) et son étude approfondie a été entreprise en 1968 (LAGIÈRE, 2-3-4).

Les nombreuses découvertes récentes de micro-organismes du type Mycoplasme dans le phloème de plantes manifestant une virescence des pièces florales ou des aberrations du développement végétatif (5 à 18) nous ont amenés à étudier au microscope électronique le phloème de cotonniers présentant ces mêmes troubles de la floraison (19).

Techniques

Des fragments de pédoncules floraux et de pétioles ont été prélevés sur des plantes saines et sur des plantes malades pour lesquelles nous avons aussi étudié le filet des étamines virescentes.

Ces pièces qui ont été fixées à la glucaraldéhyde puis au tétroxyde d'osmium ont été déshydratées par la série des alcools et du propylène puis incluses dans l'Epon. Les coupes ont été contrastées par l'acétate d'uranyle et le citrate de plomb.

Observations

Le phloème des plantes saines nous est apparu normal et tel que viennent de le décrire SHIH et CURRIER (20) pour des plantules de cotonnier.

Par contre, le phloème des plantes malades (surtout celui du filet des étamines) montre, au sein d'un tissu apparemment non désorganisé, de nombreuses cellules (C 1) bourrées de micro-organismes à structure de Mycoplasmes et des tubes criblés (C 2) en contenant également.

C 1 désigne les cellules proconductrices du phloème (21). Ces cellules qui deviendront tubes criblés par désintégration de leur cytoplasme sont reconnaissables à leur « différenciation nacrée » (21-22) et aux quelques structures cytoplasmiques qui persistent (dictyosomes, mitochondries, ribosomes, tonoplaste).

C 2 désigne des tubes criblés fonctionnels qui montrent encore à la limite de la paroi cellulosique une légère « différenciation nacrée » mais ne possèdent plus ni cytoplasme ni noyau.

Les micro-organismes observés sont des formes claires de Mycoplasmes aux contours très variables mais de texture assez homogène : au centre de la particule, une zone nucléaire avec des fibres de DNA et, à la périphérie, des ribosomes peu nombreux. La particule est limitée par une membrane cytoplasmique unitaire (pl. I, fig. C). La taille des particules voisine souvent 500 mμ.

Nous avons noté aussi l'existence de quelques cellules contenant des particules très denses aux électrons et refoulées au centre de ces cellules par une substance fibreuse développée entre la paroi cellulosique et la membrane cytoplasmique en voie de désintégration. Ces cellules peuvent être considérées comme en voie de dégénérescence. De plus, des images de Mycoplasmes (pl. III) semblant s'écouler au travers des pores de tubes criblés plus étroits que leur propre diamètre montrent, d'une part, l'extrême plasticité de ces micro-organismes et, d'autre part, l'ouverture réelle des pores, ce qui plaide en faveur d'un transport de masse au sein du phloème (20).

Conclusion

Par leur taille, leur structure interne et leur pléomorphisme, les micro-organismes que nous avons observés entrent bien dans le groupe des Mycoplasmes. La corrélation étroite qui existe entre leur présence dans le phloème des plantes atteintes et la manifestation des symptômes permet de penser qu'ils sont bien l'agent infectieux. Celui-ci serait très vraisemblablement transmis de cotonnier à cotonnier par un Jasside (Homoptère) dont l'espèce reste à préciser.

BIBLIOGRAPHIE

1. DELATTRE R., 1965. — La virescence du cotonnier. I. Recherches préliminaires. *Cot. Fib. trop.* 2, 289-294.

2. LAGIERE R., H.D. FRINKING et Soma OUATTARA, 1969. — Contribution à l'étude d'une nouvelle maladie du cotonnier : la Virescence. I. Symptômes et importance économique en Haute-Volta en 1968-69. *Cot. Fib. trop.*, 24, 3, 317-323.

3. LAGIERE R., Soma OUATTARA et H.D. FRINKING, 1969. — Contribution à l'étude d'une nouvelle maladie du cotonnier : la Virescence. II. Observations sur l'épidémiologie. *Cot. Fib. trop.*, 24, 4 (sous presse).

4. LAGIERE R. et Soma OUATTARA, 1969. — Contribution à l'étude d'une nouvelle maladie du cotonnier : la Virescence. III. Résultats d'essais de transmission de la maladie. *Cot. Fib. trop.*, 24, 4 (sous presse).

5. DOI Y., M. TERANAKA, K. YORA et H. ASSUYAMA, 1967. — Mycoplasma or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, Potato Witches broom - aster yellows of paulownia witches'broom. *Ann. Phytopath. Soc. Japan*, 33, 4, 259-266.

6. MAILLET P.L., J.P. GOURRET et C. HAMON, 1968. — Sur la présence de particules de type Mycoplasme dans le liber de plantes atteintes de maladies du type « jaunisse » (aster-yellow, phyllodie du trèfle, stolbur de la tomate) et sur la parenté ultrastructurale de ces particules avec celles trouvées chez divers Insectes Homoptères. *C. R. Acad. Sc.* Paris, 266, 2309-2311.

7. GOURRET J.P. et P.L. MAILLET, 1968. — Observation de Mycoplasmes dans des plantes malades. *C.R. Soc. Biol.*, 162, 5-6, 1205.

8. LENORMAND M. et J.P. GOURRET. — Une virescence de la giroflée *Cheiranthus Erysimum allioni. Ann. Epiphyties* (sous presse).

9. COUSIN M.Th., J.P. GOURRET, J.P. LACOTE et F. LECLANT. — Découverte de particules de type « Mycoplasme » dans le liber de *Convolvulus arvensis* récoltés dans un champ de lavandins atteints de flétrissements. *Ann. Phytopath.* (sous presse).

10. GIANNOTTI J., G. DEVAUCHELLE et C. VAGO, 1968. — Micro-organismes de type mycoplasme chez une cicadelle et une plante infectées par la phyllodie. *C. R. Acad. Sc. D.*, 266, 2168-2171.

11. J. GIANNOTTI, G. MORVAN et C. VAGO, 1968. — Micro-organismes de type mycoplasme dans les cellules libériennes de *Malus sylvestris* L. atteint de la maladie des proliférations. *C. R. Acad. Sci.*, 267, 1, 76-77.

12. GIANNOTTI J., G. MARCHOU, C. VAGO et J.L. DUTHOIT, 1968. — Micro-organismes de type mycoplasme dans les cellules libériennes de *Solanum lycopersicum* L. atteintes de stolbur. *C. R. Acad. Sci.*, 4, 454-456.

13. GIANNOTTI J., A. CAUDWELL, C. VAGO et J.L. DUTHOIT, 1969. — Isolement et purification de micro-organismes de type mycoplasme à partir de vignes atteintes de Flavescence dorée. *C. R. Acad. Sci.* Paris, 268, 5, 845-848.

14. MARAMOROSCH K., E. SHIKATA et R.R. GRANADOS, 1968. — Structures resembling Mycoplasma in diseased plants and in insect vectors. *Transactions of the New-York Academy of Sciences*, ser. II, 30, 6, 841-855.

15. GRANADOS R.R., K. MARAMOROSCH et E. SHIKATA, 1968. — Mycoplasma : Suspected etiologic agent of corn stunt. *Proc. New-York Acad. Sci.*, 60, 3, 841-844.

16. SHIKATA E., K. MARAMOROSCH, C. LING et T. MATSUMOTO, 1968. — On the mycoplasma-like structures encountered in the phloem cells of American aster yellows, corn stunt, Philippine rice yellow dwarf and Taiwan sugar cane white leaf diseased plants. *Ann. Phytopath. Soc. Japan*, 34, 2.

17. PLOAIE P., R. GRANADOS et K. MARAMOROSCH, 1968. — Mycoplasma-like structures in periwinkle plants with Crimean yellows, European clover dwarf, stolbur and parastolbur. *Phytopath.*, 58, 1063.

18. PLOAIE P. — Ultrastructure of Mycoplasma-like agents present in european yellows-type diseases. *Conférence nationale de Microbiologie générale et appliquée*, Bucarest, 4-7 décembre 1968.

19. COUSIN M.Th., P.L. MAILLET et J.P. GOURRET, 1969. — La virescence du cotonnier (*Gossypium hirsutum* L.), nouvelle maladie à Mycoplasmes. *C. R. Acad. Sci.*, série D, 268, 19, 2382-2384.

20. SHIH C.Y. et H.B. CURRIER, 1969. — Fine structure of phloem cells in relation to translocation in the cotton seedling. *Amer. J. Bot.*, 56, 4, 464-472.

21. R. BUVAT, 1963. — Les infrastructures et la différenciation des cellules criblées de *Cucurbita pepo* L. *Port. Acta Biol.*, ser. A, 7, 249-299.

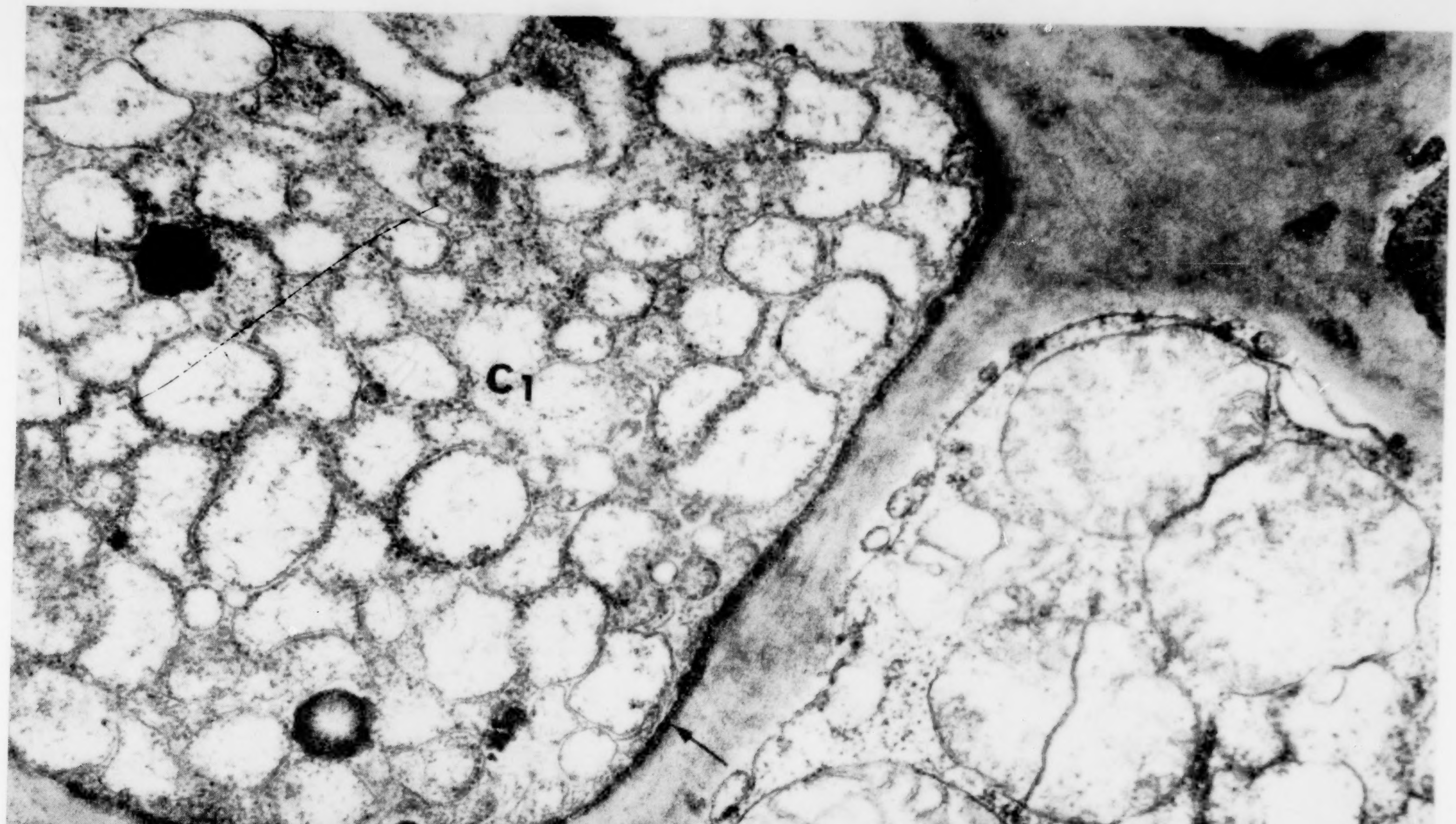
22. ROLAND J.C. et D. SANDOZ, 1969. — Détection cytochimique des sites de formation des polysaccharides prémembranaires dans les cellules végétales. *J. Microscopie*, 8, 263-268.

CONCLUSION

By their size, their internal structure and their pleomorphism the microorganisms that we have observed do fall in the Mycoplasms group. The close correlation existing between their presence in the phloem of the plants attacked and the display of symptoms permits to think that they are actually the infectious agent. The latter would very likely be transmitted from one cotton plant to another by a Jassid (Homoptera), the species of which remains to be determined.

CONCLUSIÓN

Por su talla, su estructura interna y su pleomorfismo los microorganismos que hemos observado corresponden bien al grupo de los Micoplasmas. La correlación estrecha que existe entre su presencia en el floema de las plantas atacadas y la manifestación de los síntomas, permite pensar que constituyen efectivamente el agente infeccioso. Este puede ser muy probablemente transmitido de algodonero en algodonero por un Jasside (Homóptero), cuya especie queda por precisar.

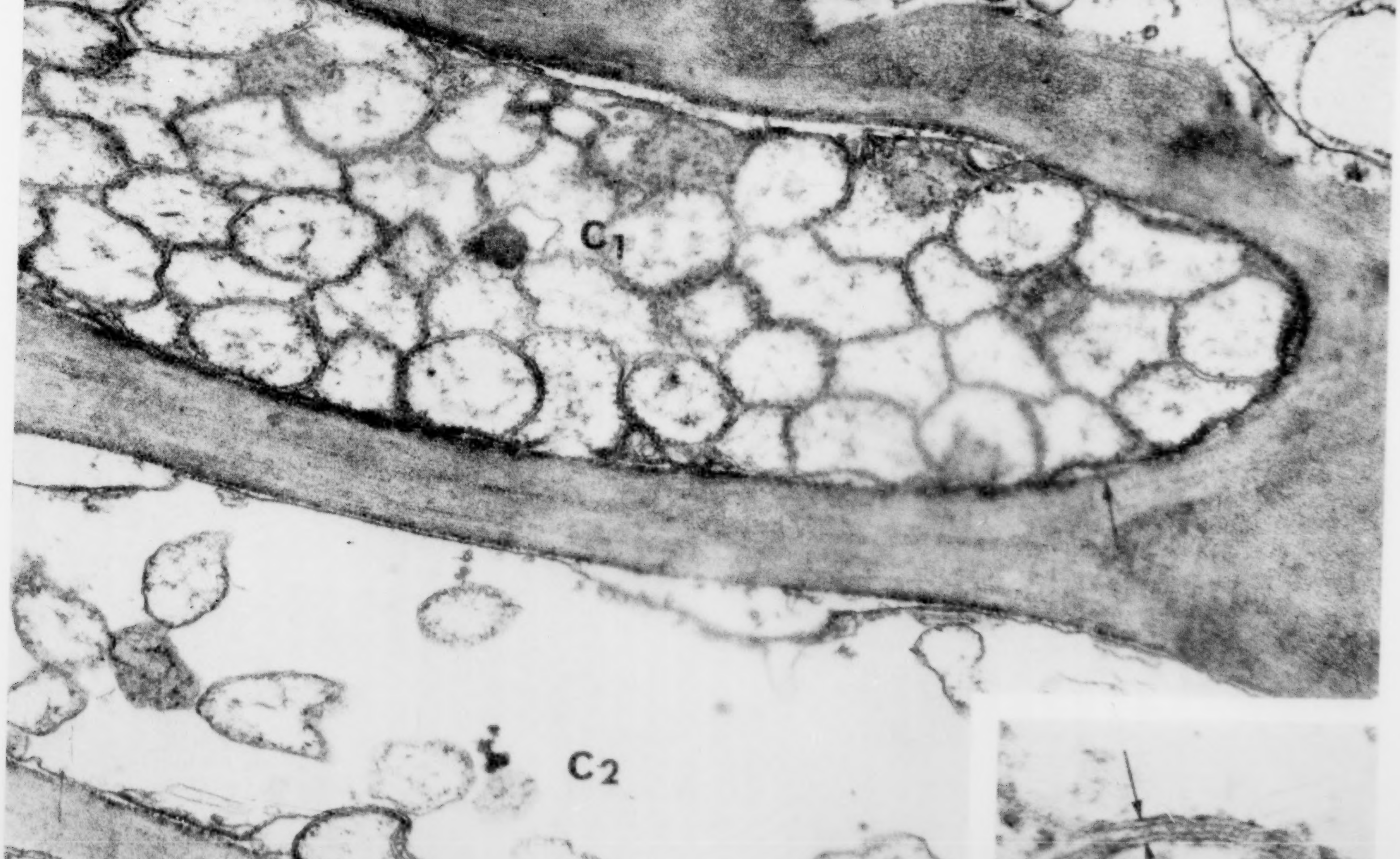


Pl. I. — Coupe[illegible] d[illegible] le [illegible] d'une étamine virescente : Mycoplasmes dans les cellules proconductrices (C 1) du [illegible]. Les flèches indiquent la « différenciation nacrée » de ces cellules (C 1).

a : Mycoplasmes de différentes tailles dans une cellule proconductrice ; entre eux des grains d'amidon (denses aux électrons) en dissociation [illegible] moins avancée ; à droite, une cellule compagne avec ses nombreuses mitochondries dont une en division. G x 30 000.

b : Mycoplasmes de taille et de texture homogènes tassés dans une cellule proconductrice (C 1) et dispersés dans un tube criblé (C 2). G x 30 000.

c : Zone d'affrontement de deux Mycoplasmes montrant la membrane unitaire qui les limite (flèches). G x 150 000.



de plus de 20 jours ne sont pratiquement plus attaquées par les chenilles de la capsule.

Le gossypol ne peut donc pas jouer un rôle dans l'attaque des capsules par les chenilles. Le niveau plus bas du taux de gossypol dans les capsules âgées des cotonniers sans glandes est par conséquent sans importance.

CONCLUSION

Les élevages sur milieu artificiel sont en général relativement faciles à réaliser. Par conséquent, il existe une tendance de chercher de plus en plus la solution des problèmes entomologiques dans des essais réalisés en laboratoire. Les résultats que nous venons de donner suggèrent que ces essais fournissent quelquefois des résultats d'une application restreinte.

Bottger et Patana (1966) ont, par exemple, déterminé les DL 90 de gossypol vis-à-vis de quelques insectes nuisibles du cotonnier ; ils trouvent une toxicité relative
les auteur
sauvages
croiser av
menter le
des insect

L'efficac
teuse dan
fructifères

Nos rés
sans glan
gossypol c
résulta' u
et par *H.*
par *Earia*

L'étude
être éclai
cotonniers

1969

FOLLIN, J.C.

Sur les différentes formes de Glomerella Spaul. et Schr. et de Colletotrichum Cda isolées du Cotonnier. I. Localisation et étude morphologique. II. Etude du pouvoir pathogène, premières conclusions. [On the different forms of Glomerella and Colletotrichum isolated from Cotton. I. Localization and morphological study. II. Pathogenicity study, first conclusions.]

Coton Fibr. trop., 24 (3):337-343; 345-350. [1 col. pl. (2 fig.), 1 fig., 6 tables; 1 pl. (5 fig.), 1 graph, 4 tables. 15 ref. Engl. P.T.O.

RESUME

En général, la littérature signale une grande sensibilité des cotonniers sans glandes vis-à-vis de plusieurs insectes, notamment *Heliothis zea*.

Des recherches effectuées pendant trois ans au Tchad ont révélé que les cotonniers sans glandes subissent des attaques plus fortes d'*Earias* spp., mais sont moins attaqués par les chenilles d'*Heliothis armigera* et de *Diparopsis watersi*. Il se trouve que l'absence du gossypol ne peut jouer un rôle dans l'attaque des capsules des cotonniers sans glandes par les chenilles de la capsule.

BIBLIOGRAPHIE

BOTTGER G.T. et R. PATANA, 1966. — Growth, development and survival of certain Lepidoptera fed gossypol in the diet. *J. econ. Ent.*, 59, 1166-1168.

BOTTGER G.T., E.T. SHEEHAN et M.J. LUKEFAHR, 1964. — Relation of gossypol content of cotton plants to insect resistance. *J. econ. Ent.*, 57, 283-285.

BRADER L., 1967. — La faune des cotonniers sans glandes dans la partie méridionale du Tchad - I - Altises. *Cot. Fib. trop.*, 22, 171-181.

GALLUP W.D., 1928. — A chemical study of the development of cotton bolls and the rate of formation of gossypol in the cotton seed. *J. agr. Res.*, 36, 471-480.

JENKINS J.N., F.G. MAXWELL et H.N. LAFEVER, 1966. — The comparative preference of insects for glanded and glandless cottons. *J. econ. Ent.*, 59, 352-356.

LUKEFAHR M.J., L.W. NOBLE et J.E. HOUGHTALING, 1966. — Growth and infestation of bollworms and other insects on glanded and glandless strains of cotton. *J. econ. Ent.*, 59, 817-820.

MAXWELL F.G., J.N. JENKINS, H.N. LAFEVER, M.J. LUKEFAHR et G.T. BOTTGER, 1965. — Insect situation on glandless cotton. A progress report. *Nat. Cott. Counc., Proc. 17th ann. Cott. Improv. Conf.*, Atlanta, Georgia, 73-74.

REEVES R.G. et J.O. BEASLEY, 1935. — The development
Res., 51, 935-944.
— Genetics aspects
and flower buds of
21.
18. — Chemistry and
cotton plant, with
nilar glands in rela-
453.

landless cotton is
several insects, in
ad for three years
ts are more seve-
s severely attacked
sis watersi *worms.*
It happens that the absence of gossypol cannot have any part in the attack of glandless cotton by boll worms.

RESUMEN

En general, la literatura señala una gran sensibilidad de los algodoneros sin glándulas con respecto a varios insectos, principalmente Heliothis zea.

Investigaciones efectuadas durante tres años en el Tchad, han puesto de manifiesto que los algodoneros sin glandulas experimentan ataques más fuertes del Earias *spp. pero son menos atacados por las orugas del* Heliothis armigera *y del* Diparopsis watersi. *Ocurre que la ausencia del gossypol no puede desempeñar ningún papel en el ataque de las cápsulas de los algodoneros sin glándulas por las orugas de la cápsula.*

SUR LES DIFFÉRENTES FORMES DE *Glomerella* Spaul. et Schr. ET DE *Colletotrichum* Cda ISOLÉES DU COTONNIER

I. - Localisation et étude morphologique

par

J. C. FOLLIN

Phytopathologiste
Station Centrale de BAMBARI

Ce travail a été réalisé en 1967 et 1968 sur la Station de BAMBARI et au Laboratoire de Biologie expérimentale de la Faculté des Sciences d'ORSAY (Service de Cryptogamie).

INTRODUCTION

L'isolement, au cours de travaux de routine, de souches de *Colletotrichum* très diverses dans leur morphologie a conduit à réaliser des isolements systématiques dans les différents organes du cotonnier à différents stades et à constituer une collection des différents types rencontrés.

Le but de ce travail était triple : tout d'abord voir s'il existe une spécialisation de certains types de souches, c'est-à-dire si la répartition est différente suivant les organes, ensuite définir l'évolution d'une population de *Glomerella* et de *Colletotrichum* en fonction de la croisance du cotonnier, enfin étudier la virulence des différentes formes en vue d'une étude génétique éventuelle du pouvoir pathogène à l'aide de formes à reproduction sexuée.

L'étude présente concerne les deux premiers points.

MATÉRIEL ET MÉTHODES

Les isolements ont été réalisés à partir de graines, de plantules, de tiges et de capsules des variétés D 9, B 50, et BJA 592 (*Gossypium hirsutum L.*). Les techniques sont les suivantes :

1. *Graines.* Les graines sont délintées à l'acide sulfurique, désinfectées au bichlorure de mercure à 0,1 % (10 mn) et mises en tubes sur culot de PDA ; ces isolements sont faits sur graines entières ou décortiquées.

2. *Plantules*

— Plantules malades : les parties atteintes sont désinfectées deux minutes au bichlorure de mercure à 0,1 % et mises en boîte de Pétri sur eau gélosée ; les colonies sont ensuite repiquées sur PDA.

— Plantules saines : les plantules sont divisées en 3 parties, racines, tiges et feuilles ; ces organes sont désinfectés soit au xylol (bain rapide) et au bichlorure de mercure (2 mn), soit au bichlorure seul et mis sur eau gélosée comme précédemment (fig. 1).

3. *Tiges.* Les tiges non décortiquées sont désinfectées au xylol (bain rapide), alcool, bichlorure de mercure (10 mn), chlorure de chaux (20 mn) et mises sur gélose en boîte de Pétri ; les tiges décortiquées et les pétioles sont seulement désinfectés au bichlorure de mercure (10 et 3 mn).

4. *Capsules.* Les isolements sont réalisés à partir de capsules momifiées, de capsules portant des lésions typiques et de capsules saines ; une partie de ces derniers isolements concerne une étude réalisée par J. CAUQUIL (résultats non publiés) sur l'infection latente du cotonnier ; ils sont faits à partir de rondelles de péricarpe, du sommet de la capsule et de l'axe central ; dans tous les cas, le milieu d'isolement est l'eau gélosée.

Les souches sont identifiées sur bouillon de pomme de terre glucosé et gélosé (PDA Difco), sur décoction de haricot de Lima gélosé (Lima bean Agar Difco) et sur bouillon d'avoine gélosé.

Les thalles sont cultivés à l'étuve à 26 °C ou à la température du laboratoire (comprise entre 23 et 30 °C).

Trois séries d'isolement ont été réalisés :

A. Isolements dans différents organes du cotonnier pris au hasard dans différents endroits, ceci dans le but de constituer une collection et d'étudier l'existence éventuelle d'une répartition suivant les organes.

B. Comparaison d'isolements réalisés sur plantules portées par un champ en ouverture et par un champ en quatrième année de culture de coton, les deux parcelles d'observations étant contiguës.

C. Evolution d'une population parallèlement à l'évolution de la plante : isolement sur feuilles cotylédonnaires, feuilles, tiges décortiquées, non décortiquées et sur capsules.

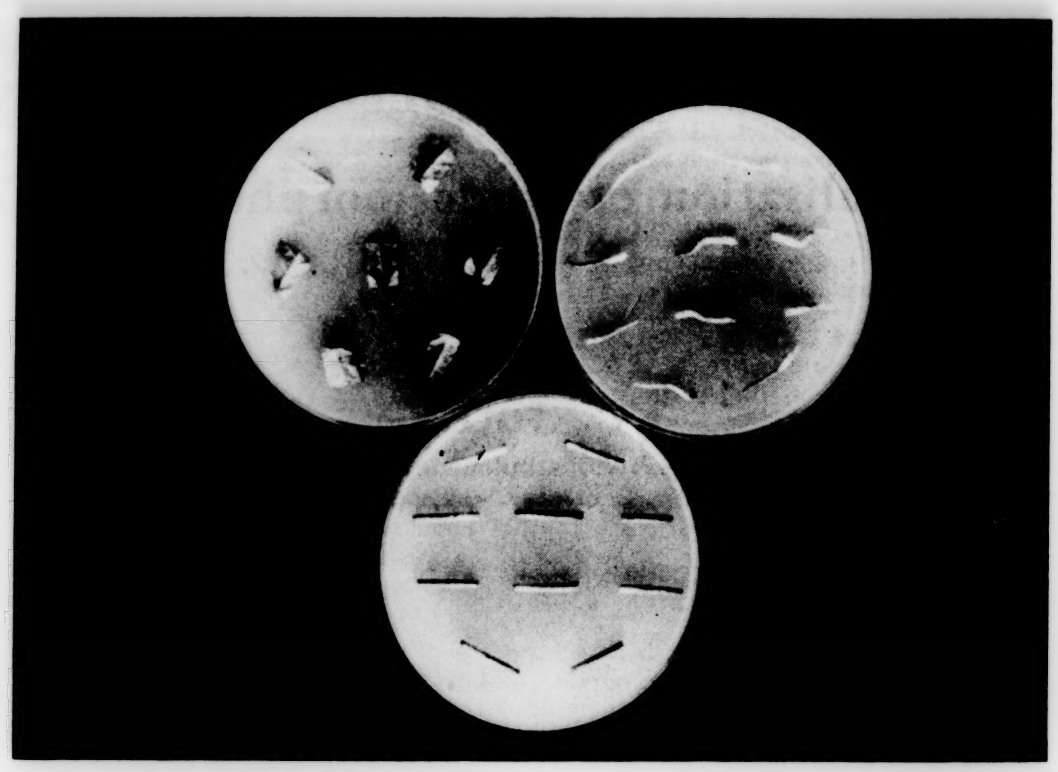


Fig. 1. — Mode d'isolement sur gélose des différentes parties d'une plantule, la technique est identique pour les capsules vertes (rondelles de péricarpe et sommets de capsules).

RÉSULTATS

Etude morphologique

Cette étude a un but pratique et non systématique ; elle reste sommaire et a été réalisée afin de dégager, par quelques traits essentiels (couleur du mycélium, mode de reproduction asexué, mensuration des spores), les groupes principaux de *Glomerella* et *Colletotrichum* parasites du cotonnier. Le classement n'est d'ailleurs pas d'une rigueur absolue et certaines souches hors types n'ont pu être classées avec certitude, le mode de reproduction asexuée est le point qui, dans les cas douteux, a décidé de la répartition.

Les caractéristiques des différentes formes sont consignées dans les tableaux I et II et illustrées par les figures 2 et 3.

En première approximation, on peut distinguer deux grandes catégories d'isolements : ceux qui ne conduisent qu'au stade *Colletotrichum* et ceux qui produisent en plus des périthèces. Cette dernière catégorie comporte deux groupes principaux : les formes *Glomerella* avec peu ou pas de conidies et les formes en produisant abondamment.

Dans la pratique, le processus de classement est le suivant :

1. Repiquage sur PDA et LBA ; les tubes sont laissés à la lumière naturelle ou artificielle, ceci permet de reconnaître les groupes I et II ; il reste les souches produisant de nombreux acervules sur LBA.

2. Ces souches restantes sont repiquées sur avoine et mises à l'obscurité, ce qui permet de distinguer les groupes III et IV.

La forme *Colletotrichum* à spores falciformes n'a pas reçu de nom d'espèce ; ses mensurations peuvent la faire considérer comme une race de *C. indicum* Dast., mais nous l'avons également observée sur Poivron (*C. annuum*) et sur Gombo (*H. esculentus*) ; il peut s'agir tout aussi bien de *C. capsici* (Syd.) Butl. et Bir.

Répartition suivant les organes

Dans tous les cas, un témoin constitué par un échantillon des graines semées au champ est mis à germer sur banc de sable stérile de façon à déterminer l'infection primaire. La forme *C. gossypii* est seule isolée et provoque généralement la mort de la plantule ; cette forme est certainement la seule à infecter les graines à l'intérieur.

Le tableau III donne les résultats principaux. Les séries C, D et H ont été réalisées sur plantules d'un champ portant des cotonniers pour la troisième année consécutive. Les organes sont désinfectés de deux manières : au bichlorure de mercure (série C et H), au xylol, puis au bichlorure de mercure (série D).

Sur plantules nécrosées et dans les graines, on ne trouve que la forme imparfaite typique *C. gossypii* South., sur lésions de capsules et sur capsules momifiées, par contre, les deux formes sont trouvées, la forme *Glomerella* plus fréquemment que la forme imparfaite comprenant *C. gossypii* et la forme *Colletotrichum* à spores falciformes.

PLANCHE I

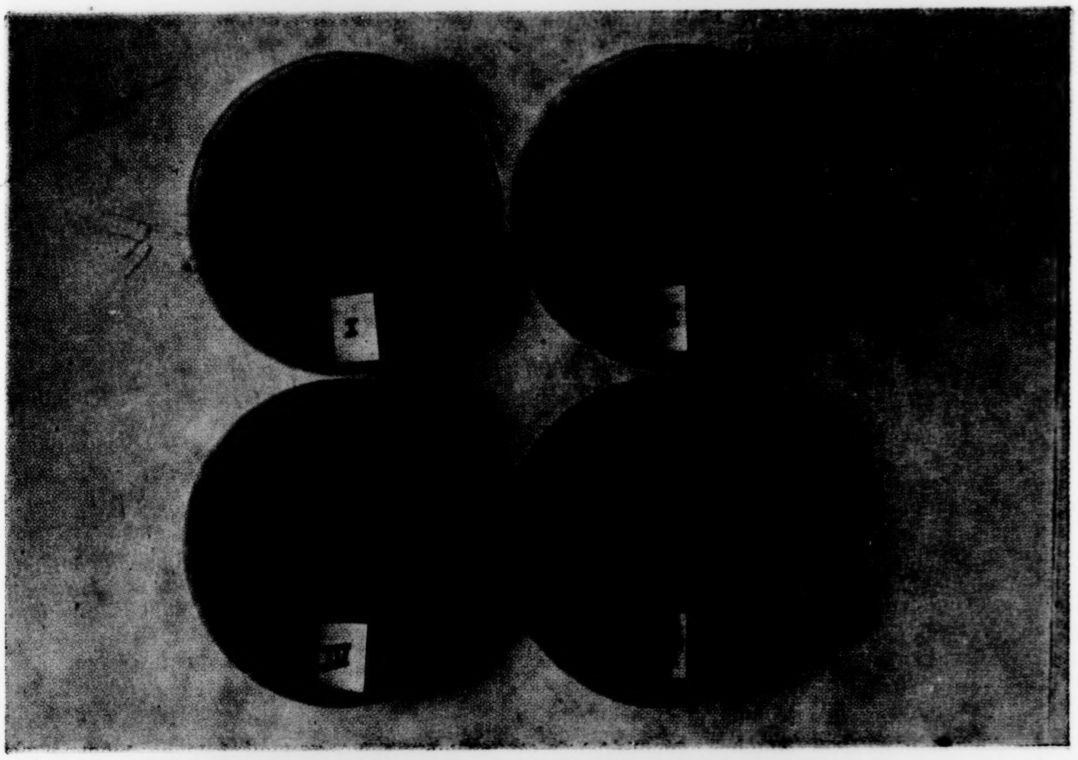


Fig. 2. — Aspect des quatre formes de *Glomerella* sp. sur milieu P.D.A.

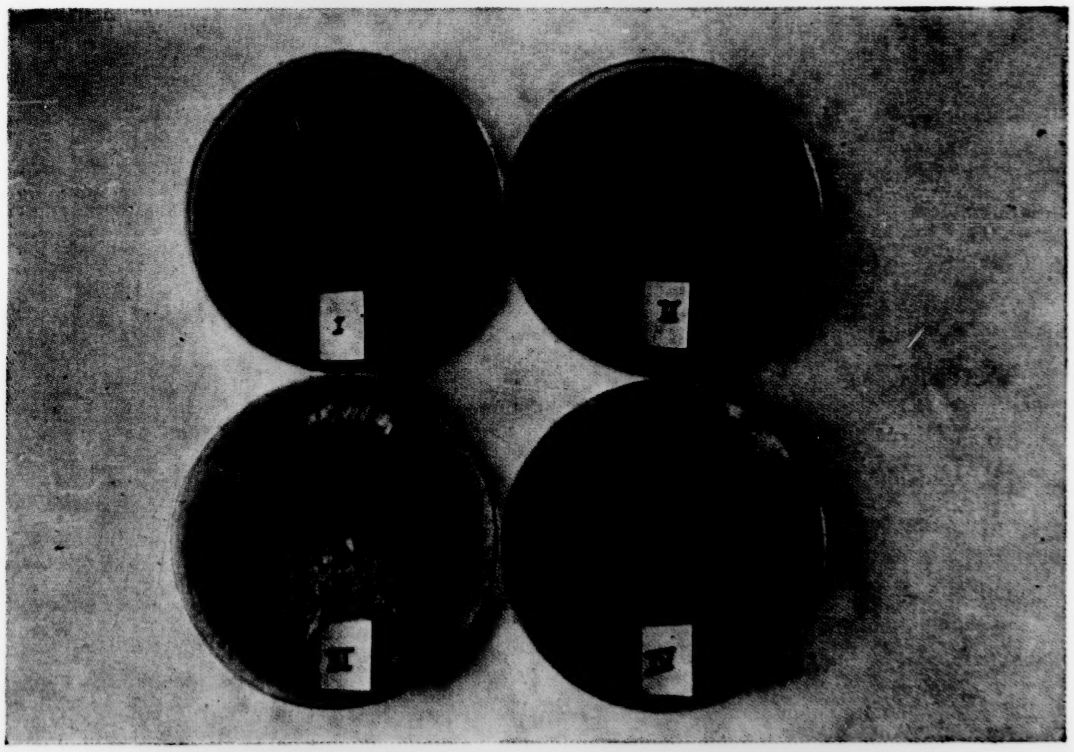


Fig. 3. — Aspect des quatre formes de *Glomerella* sp. sur milieu avoine.

Tableau I. — *Caractéristiques essentielles des différentes formes de* Glomerella *isolées du cotonnier et de* Colletotrichum gossypii *South.*

	Mycélium	Forme imparfaite	Forme parfaite	Forme des spores
Glomerella Groupe I	PDA : Myc. aérien gris acier, parfois reflet orangé. LBA : Myc. ras, blanc terne. Avoine : Myc. ras, gris pâle.	Conidies rares, cependant présence d'acervules avec des soies bien différenciées (3 à 4 par acervule).	PDA : périthèces en glomérules. LBA : périthèces en glomérules. Avoine : per. en glomérules et par groupe de 4 à 5.	Conidies cylindriques arrondies aux deux extrémités. I présente parfois des conidies apiculées.
Groupe II	PDA : Myc. aérien blanc à jaune milieu ocre jaune à orange. LBA : Myc. ras blanc terne. Avoine : Myc. ras gris pâle.	Identique à I.	PDA : identique à I. LBA : glomérules plus abondants que I. Avoine : identique à I.	Ascospores arquées en forme de croissant. Les spores de IV, à largeur plus faible sont de forme plus élancée.
Groupe III	PDA : Myc. aérien vert clair puis foncé. LBA : myc. ras blanc. Avoine : myc. blanc couvert d'acervules.	Sporulation abondante à la lumière et à l'obscurité. Acervules sans soies.	PDA : per. en glomérules. LBA : glomérules et per. isolés. Avoine : glomérules rares.	
Groupe IV	PDA : myc. aérien vert foncé. LBA : myc . ras blanc terne. Avoine : myc. ras gris acier.	Acervules sans soies et conidies abondantes dans les cultures exposées à la lumière.	PDA : per. en glomérules. LBA et avoine : glomérules à l'obscurité, glomérules et perithèces isolés à la lumière.	
Colletotrichum gossypii	PDA : myc. aérien vert foncé à noir. LBA : myc. ras blanc. Avoine : myc. ras, gris pâle à gris acier.	Sporulation abondante surtout sur LBA et avoine, présence de soies irrégulières sur LBA.		Conidies ovoïdes à piriformes arrondies au bout, droites, souvent apiculées à la base et étranglées au milieu.

Tableau II. — *Dimensions en microns des spores de* Glomerella *et* Colletotrichum *isolés de cotonniers (le milieu de culture est constitué par une décoction gélosée de flocons d'avoine).*

	Conidies	Ascospores
Glomerella I	13,0 × 3,5 (9,0-15,0) × (3,0-4,5)	15,9 × 4,2 (14,4-19,2) × (3,6-4,8)
II	—	15,1 × 4,0 (13,8-17,4) × (3,6-4,8)
III	12,1 × 3,5 (10,2-15,4) × (3,0-4,0)	15,1 × 3,6 (13,2-17,6) × (2,2-4,2)
IV	11,5 × 3,3 (10,2-14,4) × (3,0-3,6)	15,2 × 3,5 (13,2-18,0) × (2,4-4,2)
Colletotrichum gossypii	14,4 × 3,1 12,0-19,8) × (2,4-4,2)	—
Colletotrichum sp.	12,6 × 3,2 (10,2-15,0) × (3,0-3,6)	—
Colletotrichum à spores falciformes	23,0 × 2,9 (26-20,4) - (2,4-3,0)	—

Tableau III. — *Répartition des différentes formes de* Glomerella *et de* Colletotrichum *en fonction du lieu d'isolement.*

Lieux d'isolement	Nombre d'isolements	Forme imparfaite	Forme parfaite et f. p. + f. imp.
Graines			
décortiquées	60	1	—
non décortiquées	60	3	—
Plantules à racines nécrosées (lésions typiques d'anthracnose)	19	19	—
Plantules saines			
Série C :			
racines	130	5	2
tiges	130	3	—
feuilles	130	5	20
Série D :			
racines	130	1	—
tiges	130	2	—
feuilles	130	1	—
Série H : feuilles	130	6	15
Tiges - Infection latente	400	—	4
Capsules			
Série L :			
lésions typiques d'anthracnose	14	4	10
Série M :			
capsules momifiées	30	3	5
Série N :			
infection latente de la capsule	100	1	35

En infection interne, c'est la forme parfaite qui est le plus souvent isolée ; rare dans les tiges, elle est particulièrement abondante dans les feuilles et les téguments des capsules.

Importance suivant le précédent cultural (tableau IV)

Deux séries d'isolements sur cotonniers portés par un champ en ouverture montrent l'augmentation de la population de *Glomerella* dans le sol ; celle-ci passe de 36 %, par rapport au nombre de champignons isolés, à 78,6 % dans le champ en quatrième année de culture. Par contre, la situation des formes *Colletotrichum* n'évolue pas.

Evolution dans la plante (tableau V)

Ces résultats confirment les précédents. La population de *Glomerella* est particulièrement abondante en infection interne des capsules et des feuilles ; elle est plus rare dans les tiges et limitée aux parties non encore lignifiées. Le vieillissement des tissus favorise d'autres champignons, en particulier des *Phoma* et un *Phomopsis* sp. très fréquemment isolé.

Dans les parties non encore lignifiées, l'infection reste superficielle comme le montrent les isolements réalisés à partir de pétioles.

Tableau IV. — *Fréquence d'isolement des formes* Glomerella *et* Colletotrichum *de plantules (feuilles cotylédonnaires) portées par un champ en ouverture et par un champ cultivé en coton pour la quatrième année consécutive.*

	Champ en ouverture	Champ en 4e année
Nombre d'isolements	170	170
Nombre d'isolements positifs	38	61
Nombre de *Glomerella* et de *Colletotrichum*	14	48
Groupe I	6	17
Groupe II	0	12
Groupe III	2	11
Groupe IV	5	6
forme imparfaite typique	1	2
% *Glomerella* par rapport au nombre d'isolements	8,2	28,2
% *Glomerella* par rapport au nombre d'isolements positifs	36,8	78,6

Tableau V. — *Répartition des différentes formes parfaites et imparfaites en fonction de l'âge du cotonnier.*

Lieux d'isolement	Nombre d'isolements réalisés	Nombre d'isolements positifs	Forme parfaite et f.p. + f. imparfaite	F. imparfaite
Plants de 10 jours				
Feuilles cotylédonnaires	170	61	46	2
Plants de 4 semaines				
Derniers entrenœuds	60	9	2	—
Dernières feuilles (6ᵉ)	125	12	3	—
Plants de 2 mois				
Feuilles vers le sommet	90	50	41	—
Pétioles	90	13	7	—
Plants de 2,5 mois				
Bas de tiges	45	43	1	—
Bas de tiges décortiquées	45	8	—	—
Avant-derniers entrenœuds	45	19	7	—
Plants de 3 mois				
Capsules				
• Sommets	100	48	29	4*
• Pédoncules	50	38	21	—
• Péd. décortiqués	50	16	—	—

* 1 souche à spores falciformes.

Tableau VI. — *Répartition dans les groupes I, II, III et IV des différentes souches de* Glomerella *observées.*

Lieux d'isolement	Nombre de souches observées	Groupe I %	Groupe II %	Groupe III %	Groupe IV %
Feuilles cotylédonnaires	72	47,2	23,6	16,6	12,6
Feuilles	40	27,5	62,5	5,0	5,0
Tiges non aoutées et pétioles de feuilles	14	35,7	42,8	—	21,5
Capsules :					
Sommets	63	30,3	23,8	19,0	26,9
Pétioles	21	19,0	62,0	—	19,0
Capsules momifiées et capsules avec lésions externes	15	26,6	—	46,8	26,6

Les formes *Colletotrichum* sont isolées beaucoup plus rarement et seulement de plantules et de capsules.

Répartition des différentes formes de *Glomerella* (tableau VI)

En infection interne, ce sont les formes I et II qui sont le plus fréquemment isolées ; par contre, la situation est inversée lorsqu'il s'agit d'isolements à partir de capsules lésées ou de capsules momifiées, ce sont alors les formes possédant la forme imparfaite et sporulant abondamment (groupes III et IV) qui prédominent.

DISCUSSION

Une première constatation est qu'il n'y a pas de répartition particulière suivant les organes : des formes parfaites et imparfaites sont isolées de racines, de tiges et de capsules ; par contre, on peut constater une spécialisation particulière ; en effet, dans les cas de lésions typiques sur plantules et à l'intérieur des graines, on isole toujours la forme imparfaite typique de l'anthracnose, de même que dans le cas d'isolement sur plantules désinfectées très sévèrement par un bain dans le xylol qui détruit les organismes d'une façon plus profonde que le traitement au bichlorure de mercure. Il semble

donc pour les plantules que *C. gossypii* soit spécifique des nécroses et des infections internes profondes, infection interne ou début d'attaque encore invisible à l'œil nu.

Des plantules désinfectées au bichlorure de mercure, on isole toutes les formes, les formes *Glomerella* correspondraient à une infection interne superficielle. Des plants plus âgés, on n'isole ces formes que des parties non encore lignifiées, et il est probable non seulement qu'elles sont incapables de franchir le cambium lorsque la structure est secondaire, mais qu'elles restent également superficielles dans les parties vertes comme le montrent les isolements à partir de pétioles.

Dans le cas des capsules, la situation est différente. Il est en effet très courant d'observer la forme parfaite sur des lésions typiques d'anthracnose, ce qui ne démontre d'ailleurs pas le caractère de parasite primaire, les capsules pouvant être soumises à des déprédations multiples (chenilles, piqûres d'Hémiptères...). Par ailleurs, le grand nombre de formes *Glomerella* isolé des téguments de la capsule en général et de ceux de l'apex en particulier indique qu'il existe là une population parasite prête à entrer dans la capsule au moindre défaut d'étanchéité et à y provoquer une pourriture.

Enfin, la fréquence des formes *Glomerella* dans les isolements réalisés à partir de feuilles cotylédonnaires et leur multiplication avec la culture du cotonnier montrent que celles-ci peuvent assurer leur survie dans le sol d'une année sur l'autre.

CONCLUSION

Cette étude montre la grande diversité des formes de *Glomerella* et de *Colletotrichum* isolées du cotonnier mais reste descriptive et soulève deux questions fondamentales :

1. Quelles sont les formes pathogènes et les formes seulement parasites ?

2. Existe-t-il une filiation entre les différents types isolés ? En particulier, la forme typique *C. gossypii* résulte-t-elle de l'évolution d'une des formes *Glomerella* décrites ?

Une étude du pouvoir pathogène est nécessaire pour compléter ce travail en essayant d'apporter une réponse à ces deux questions.

RÉSUMÉ

Des isolements réalisés dans les différents organes du cotonnier indiquent l'existence d'une grande diversité de forme de *Colletotrichum* : la forme *C. gossypii* South., une forme à petites spores cylindriques, une forme à spores falciformes, et de *Glomerella* : quatre grands groupes dont deux possédant en plus la forme imparfaite et très voisins macroscopiquement de *C. gossypii* mais à conidies plus courtes (groupes III et IV).

La répartition est très différente suivant les organes et peut se résumer ainsi :

Graines

(Infection interne) : *C. gossypii* South.

Plantules

— Nécrosées : *C. gossypii*.

— Saines : *Glomerella* sp. (groupe I et II surtout) 70 à 95 % ; *C. gossypii* 5 à 20 % ; *Colletotrichum* sp. 0 à 10 %.

Plants

— Parties lignifiées : Isolement rare d'une quelconque de ces formes.

— Parties vertes (feuilles, pétioles, tiges non aoutées) : *Glomerella* sp. (groupes I et II surtout).

Capsules

— Infection interne des téguments : *Glomerella* sp. (groupes I et II surtout) ; *C. gossypii* plus rarement ; *C.* à spores falciformes : très rarement.

— Capsules momifiées et capsules présentant une pourriture externe : *Glomerella* sp. (groupes III et IV surtout) ; *C. gossypii ; C.* à spores falciformes . plus rarement.

Cette étude soulève deux problèmes importants : celui du pouvoir pathogène des différents organismes isolés et la filiation éventuelle de *C. gossypii* avec certains types de *Glomerella*.

SUMMARY

Some isolations realized from various cotton organs indicate the existence of a great diversity of Colletotrichum *forms : the* C. gossypii *South. form, a form with cylindrical small spores, a form with falciform spores, and* Glomerella *: four large groups two of which possessing besides the imperfect form and macroscopically very closely related with* C. gossypii *but with shorter conidia (groups III and IV).*

The distribution is very different according to the organs and can be summarized as follows.

Seeds

(Internal infection) : C. gossypii *South.*

Seedlings

— Necrosed : C. gossypii.

— Healthy : Glomerella *sp. (especially groups I and II) 70 to 95 %* ; C. gossypii 5 *to 20 %* ; *Colletotri*chum *sp. 0 to 10 %.*

Plants

— Lignified parts : Rare isolation of anyone of these forms.

— *Green parts (leaves, petioles, non lignified stems) :* Glomerella *sp. (especially groups I and II).*

Bolls

— *Internal infection of teguments :* Glomerella *sp. (especially groups I and II) ;* C. gossypii *more rarely ;* C. *with falciform spores : very rarely.*

— *Mummified bolls and bolls displaying external rot :* Glomerella *sp. (especially, groups III and IV) ;* C. gossypii ; C. *with falciform spores : more rarely.*

This study sets two important problems : that of the pathogenic power of various isolated organisms and the eventual filiation of C. gossypii *with some* Glomerella *types.*

RESUMEN

Aislamientos realizados en los diferentes órganos del algodonero indican la existencia de una gran diversidad de forma de Colletotrichum *: la forma* C. gossypii *South., una forma con pequeñas esporas cilíndricas, una forma con esporas falciformes, y de* Glomerella *: cuatro grandes grupos de los cuales dos poseen además la forma imperfecta y muy próximos macroscópicamente de* C. gossypii, *pero con conidios más cortos (groupos III y IV).*

La repartición es muy diferente según los órganos y puede resumirse así :

Simientes

(Infección interna) : C. gossypii *South.*

Plántulas

— *Con necrosis :* C. gossypii *South.*

— *Sanas :* Glomerella *sp. (Grupo I y II sobretodo) 70 a 95 % ;* C. gossypii *5 a 20 % ;* Colletotrichum *sp. 0 a 10 %.*

Plantas

— *Partes lignificadas : Aislamiento raro de una cualquiera de esas formas.*

— *Partes verdes (hojas, peciolos, tallos no madurados) :* Glomerella *sp. (Grupo I y II sobretodo).*

Cápsulas

— *Infección interna de los tegumentos :* Glomerella *sp. (Grupo I y II sobretodo) ;* C. gossypii *más raramente ;* C. *a esporas falciformes : muy raramente.*

— *C. momificados y C. presentando una podredumbre externa :* Glomerella *sp. (Grupo III y IV sobretodo) ;* C. gossypii ; C. *a esporas falciformes : más raramente.*

Este estudio plantea dos problemas importantes : el del poder patógeno de los diferentes organismos aislados y la filiación eventual de C. gossypii *con ciertos tipos de* Glomerella.

SUR LES DIFFÉRENTES FORMES DE *Glomerella* Spaul. et Schr. ET DE *Colletotrichum* Cda ISOLÉES DU COTONNIER *(Gossypium hirsutum* L.*)*

II. - Etude du pouvoir pathogène, premières conclusions

par

J. C. FOLLIN

Phytopathologiste

Station Centrale de BAMBARI

Ce travail a été réalisé en 1968 sur la Station de BAMBARI.

INTRODUCTION

Une étude précédente (5) a montré que plusieurs formes de *Glomerella* et de *Colletotrichum* coexistaient sur le cotonnier sinon avec des répartitions, tout au moins, semble-t-il, avec des spécialisations parasitaires particulières. Cette étude amenait à poser le problème des formes parasites et des formes pathogènes ainsi que celui de la filiation entre les différents types. C'est dans cette optique que fut réalisée une série d'inoculations expérimentales sur plantules et sur capsules à partir de souches représentant les différentes formes isolées.

MATÉRIEL ET MÉTHODE

A) Infection des plantules

1 - *Infection sur banc de sable*

Les graines délintées à l'acide sulfurique puis triées sont immergées 6 h dans une suspension dans l'eau stérile de spores et de mycélium (broyage ménagé de 2 tubes à essai dans 200 cc d'eau); elles sont ensuites repiquées sur banc de sable stérile (fig. 1). L'observation des résultats se fait 6 jours après le semis. Cette méthode est utilisée pour avoir une réponse rapide sur l'existence ou la non-existence de pouvoir pathogène d'une souche et pour tester les réactions variétales de cotonniers à l'anthracnose. Dans ce dernier cas, l'appréciation des résultats est faite en calculant la mortalité réelle par la correction d'Abott :

$$Mr = \frac{100\ (Mo - Mt)}{100 - Mt}$$

Mr = mortalité réelle ;
Mo = mortalité observée ;
Mt = mortalité du témoin.

2 - *Infection en tubes*

Les graines délintées à l'acide sulfurique sont triées, désinfectées au bichlorure de mercure à 0,1 % pendant 10 minutes puis mises à germer 24 heures entre deux couches de coton hydrophile imbibé d'eau stérile. Les graines ayant normalement germé sont repiquées en tube sur milieu gélosé (a).

L'infection est faite lorsque les plantules ont 6 jours en ajoutant 1 ml d'une suspension de spores et de mycélium ; les résultats sont appréciés en calculant chaque jour les pourcentages cumulés de plants morts sur 30 plants inoculés (fig. 2).

B) Infection de capsules

1 - *Infection par pulvérisation*

Les capsules sont soit mises en bocaux (fig. 3) (8), soit fixées après désinfection pendant 5 mn au bichlorure de mercure sur un plateau en grillage maintenu au-dessus d'un bac rempli d'eau et entouré d'une gaine en polyéthylène permettant de garder une humidité relative voisine de 100 % (fig. 4). L'infection est faite par pulvérisation de spores sur les capsules.

2 - *Infection par piqûres*

Elle est réalisée sur capsules au champ suivant la méthode utilisée depuis plusieurs années sur la station de BAMBARI (1). L'appréciation des résultats est faite en examinant après 8 jours chaque loge et en les classant dans une des quatre catégories :

0 : loge saine ;
1 : loge peu attaquée ;
2 : loge moyennement attaquée ;
3 : loge totalement détruite.

(a) Il s'agit de la solution de crone gélosée (14) dont la composition est la suivante : $K_2H\ PO_4$: 1 g - KCl : 7 g - $CaSO_4$: 2,5 g - $MgSO_4\ 7H_2O$: 2,5 g - $Ca_3\ (PO_4)_2$: 0,5 g - $FePO_4$: 0,5 g - KNO_3 : 2 g ; les sels sont mélanges dans un mortier, la solution est préparée en mettant 1,5 g de ce mélange dans 1 l d'eau, la gélose est ajoutée (20 g/l) et le tout autoclavé.

Après avoir fait la somme des chiffres obtenus pour chaque loge dans les capsules étudiées, on divise par le nombre de loges pour avoir le degré de symptômes internes ; ce résultat est complété par le calcul du nombre de loges attaquées divisé par le nombre de capsules inoculées, il traduit la faculté pour un parasite de passer d'une loge à l'autre.

C) Souches utilisées

D'une manière générale, on préfère prendre des souches présentant une forme imparfaite, ceci permettant un inoculum plus homogène.

1 - *Inoculation des plantules*

— Sur sable : tous les types de *Glomerella* et *Colletotrichum* rencontrés.

— En tubes : *Glomerella*, 1 souche de chacun des groupes I, II, III et IV, la souche IV a été inoculée sous sa forme (+) et sa forme mutante (—) (4, 6).

Colletotrichum, souche typique et souche à spores falciformes.

2 - *Inoculation des capsules*

— Par pulvérisation : *Colletotrichum gossypii* et forme IV de *Glomerella* (isolée en 1967 de lésions typiques sur capsules).

— Par piqûres : 1 souche des groupes I, II, III et IV et 1 souche de *C. gossypii*.

Toutes les souches utilisées proviennent de clones d'origine monospore.

D) Terminologie

Le mot parasite est employé dans son sens strict, c'est-à-dire qu'il définit un organisme capable de vivre aux dépens d'un hôte vivant, le mot pathogène désigne un parasite pouvant déterminer une maladie, la virulence est une faculté commune aux parasites et aux pathogènes mais à des degrés différents. Le pouvoir pathogène est l'apanage des organismes pathogènes.

RÉSULTATS

Inoculation des plantules

1) Les inoculations par trempage des graines puis semis sur sable donnent des résultats nets : les souches imparfaites typiques à spores élancées produisent des infections très fortes. Une étude en cours sur les réactions variétales donne une idée des mortalités couramment obtenues (tableau 1). Les formes parfaites donnent toujours, par contre, des résultats négatifs même en utilisant des temps de trempage plus longs et en les complétant par arrosage de spores en suspension dans l'eau. Les formes imparfaites à spores courtes et à spores falciformes ont également donné des résultats négatifs.

Tableau 1. — *Mortalité obtenue par inoculation de* C. gossypii *sur différentes variétés de* Gossypium hirsutum

Variétés	Mortalité réelle %
D 9	88,4
B 50	78,0
BJA 592	69,5
Allen 333	88,4
BTK 12	85,7
SW 296	75,4
HG 9	93,7

Nous n'avons pas eu de problèmes avec la conservation du pouvoir pathogène des souches ; la souche de référence isolée de graines conserve une virulence après 1 an de repiquages successifs sur décoction de haricot de Lima gélosé (lima bean agar Difco). Pour obtenir des infections fortes, il suffit de prendre la précaution d'utiliser des cultures jeunes de 8-10 jours et d'assurer une humidité suffisante.

2) Les inoculations sur plantules en tube sur milieu gélosé ont permis de chiffrer ces différences dans la virulence des différentes souches. Après le repiquage, les cotonniers ont une croissance normale pendant 5 à 6 jours puis restent ensuite bloqués au stade deux feuilles ; la limite de cet arrêt de croissance semble ne dépendre que du milieu qui peu à peu se dessèche. Une étude réalisée dans le but de mettre cette technique au point a montré que l'on pouvait conserver ainsi ces plantules sans dommage pendant 2 mois.

— Dans ces conditions tout à fait artificielles, il est bien évident que la plante ne peut compenser par sa croissance les dégâts causés par un organisme parasite et que des souches non pathogènes dans les conditions naturelles peuvent provoquer des symptômes morbides. Le temps nécessaire pour entraîner cette mortalité nous sert alors de critère pour différencier les souches.

Tableau 2. — *Temps minimum pour obtenir* 50 % *de mortalité* (*TL* 50) *et intervalle entre le début de l'attaque et les* 100 % *de mortalité*

Souches	T.L. 50 (jours)	Int. (0-100) (jours)
C. gossypii	7	4
Glomerella		
I	18	8
II	17	8
III	18	8
IV +	18	7
IV —	19	9
C. à spores falciformes	20	11

PLANCHE I

Fig. 1. — Plantules issues de graines infectées par *C. gossypii* (à droite) et plantules témoins.

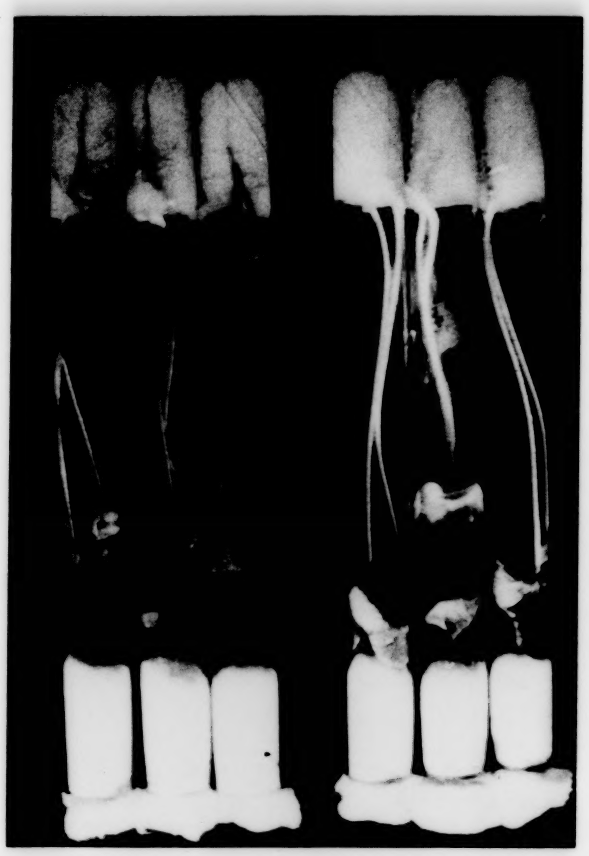


Fig. 2. — Infection de plantules en tubes - à droite infection par *C. gossypii*, à gauche, plantules témoins.

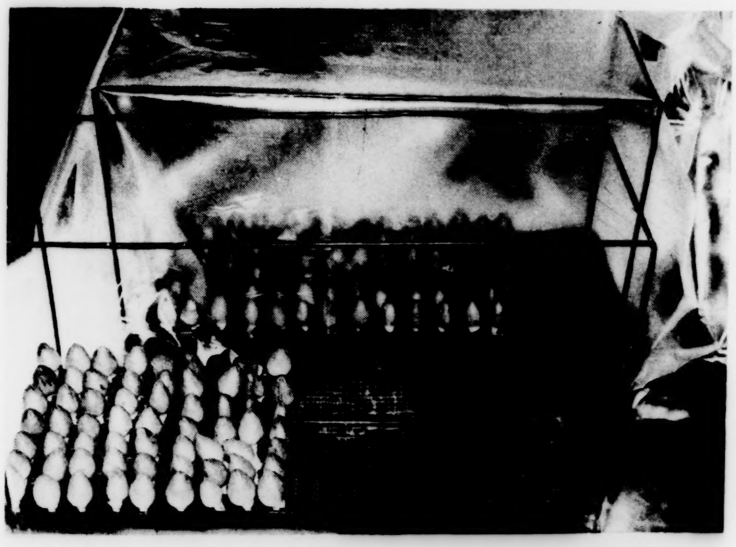


Fig. 4. — Capsules en bacs recouverts de polyéthylène infectées par pulvérisation d'une solution de spores.

Fig. 3. — Capsule infectée au laboratoire par pulvérisation d'une solution de spores.

A gauche, le micropulvérisateur Vaast.

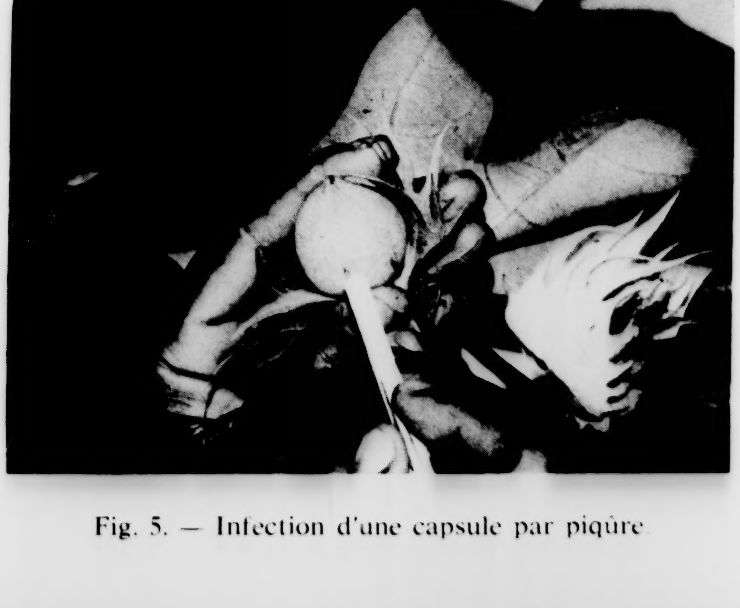
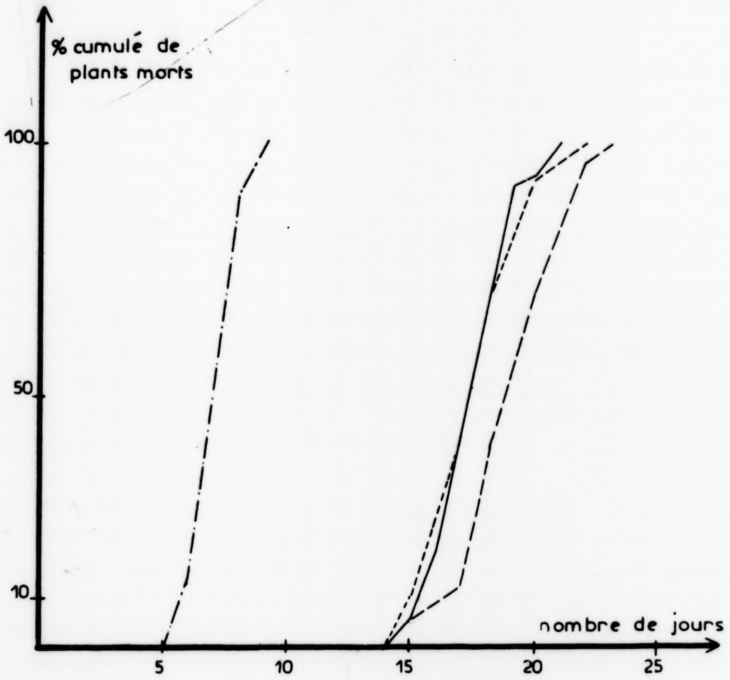


Fig. 5. — Infection d'une capsule par piqûre

— Le tableau 2 et le graphique n° 1 confirment les résultats des inoculations sur banc de sable. Seule la forme typique *C. gossypii* cause une mortalité précoce et rapide ; aucune plantule du témoin ne s'est flétrie pendant toute la durée de cette expérience. Dans ces conditions artificielles, la virulence de la forme imparfaite typique est nettement plus forte.



Graphique 1. — Courbes représentatives des pourcentages cumulés de mortalité causée par *C. gossypii* (— - —) et deux souches de *Glomerella* dont une sous la forme (+) (——) et sous la forme (—) (- - - -).

Inoculation des capsules

1) *Par pulvérisation.* La méthode isolant les capsules en bocal est la meilleure et a donné 100 % d'infection avec la souche typique et 0 % avec la forme *Glomerella*, mais le faible nombre de capsules pouvant être inoculé limite la valeur des résultats ; la méthode en bacs recouverts de polyéthylène confirme ces derniers. Il est à noter que la forme *Glomerella* n'attaque pas les capsules même blessées superficiellement (tableau 3), sa présence au champ sur lésions de capsules résulte vraisemblablement d'une blessure profonde permettant à ces organismes présents dans les téguments de pénétrer à l'intérieur de la capsule et de provoquer une pourriture interne puis une pourriture externe qui se traduit par une lésion typique d'anthracnose couverte d'acervules.

2) *Par piqûres.* Les résultats (tableau 4) montrent qu'après 9 jours d'incubation les quatre souches de *Glomerella* ont à peine endommagé plus d'une loge alors que *C. gossypii* a détruit entre 2 et 3 loges. Par piqûre également, les dégâts provoqués par la forme imparfaite typique sont plus importants.

DISCUSSION

Dans une étude précédente (5), nous posions en conclusion 2 questions :

— Quelles sont les formes pathogènes et les formes seulement parasites ?

— Existe-t-il une filiation entre les différents types de *Glomerella* et de *Colletotrichum* isolés ?

En ce qui concerne la première question, la réponse est nette : *Colletotrichum gossypii* South. est seul pathogène, les formes *Glomerella* et la forme *Colletotrichum* à spores falciformes doivent être considérées comme des parasites secondaires ce qui ne diminue d'ailleurs pas leur importance car elles sont fréquemment trouvées sur lésions typiques externes de capsules. Cette infection est très certainement consécutive à une blessure accidentelle, nous pensons surtout aux déprédations d'insectes en particulier aux dégâts des *Dysdercus* ; en définitive, pour le cas particulier des capsules, le milieu extérieur est plus important que les capacités du champignon parasite lui-même.

Les différences dans les aptitudes parasitaires des différentes formes suggèrent par ailleurs une explication aux déboires relevés dans certaines études (2) lorsque des souches infectant le cotonnier à l'état latent sont utilisées pour des inoculations artificielles de plantules. Ce sont en effet des formes *Glomerella* dans la très grande majorité des cas que l'on isole des parties internes et, sans une certaine habitude, la confusion est facile entre certaines formes parfaites (groupes III et IV en particulier) et la forme imparfaite typique.

Cette situation présente une analogie certaine avec celle de l'anthracnose du caféier. A ce sujet, deux séries d'études sont particulièrement intéressantes : celles de NUTSMAN et ROBERT (12) et celle plus récente de HOCKING (9). Leurs conclusions sont identiques sur les distinctions entre les différentes souches isolées : une forme *Colletotrichum*, *Colletotrichum coffeanum* Nov. dont le stade parfait n'a jamais été observé provoque l'anthracnose typique (coffee berry disease), entre autres cette forme est seule capable d'attaquer les baies vertes ; une autre forme également nommée *Colletotrichum coffeanum*, différente d'après HOCKING en culture, possède une forme parfaite *Glomerella* fréquemment obtenue, elle possède une virulence très faible, ses attaques se limitent aux baies mûres et aux tissus moribonds (Brown blight disease), elle peut infecter les feuilles à l'état latent sans provoquer de nécroses.

Par ailleurs, RAYNER (13) mentionne l'existence en infection latente de deux types de *Colletotrichum* : un type à spores courtes trouvé généralement sur les rameaux et les feuilles, non pathogène, et un type à spores plus longues fréquemment isolé de baies vertes, pathogène ; c'est à ce dernier type que RAYNER rattache l'agent du coffee disease.

Une situation semblable se rencontre dans le cas

Tableau 3. — *Pourriture externe obtenue par pulvérisation de spores de* C. gossypii *et* Glomerella *sur capsules de cotonnier*

	Capsules en bocaux		Capsules en bacs			
	Capsules entières		Capsules entières		Capsules blessées superficiellement	
	Nombre de capsules	% de C. pourries	Nombre de capsules	% de C. pourries	Nombre de capsules	% de C. pourries
Glomerella	6	0	63	0	70	0
C. gossypii	6	100	56	21,4	56	100
C. à spores falciformes	—	—	50	0	50	0
Témoin	—	—	49	0	38	0

Tableau 4. — *Degré moyen d'attaque et nombre de loges pourries par capsules résultant de l'inoculation de* Glomerella *sp. et* C. gossypii - 2 x 100 *capsules sont inoculées par souche*

Souches	Incubation de 9 jours		Incubation de 15 jours	
	Degré moyen d'attaque	Nombre de loges pourries par capsule	Degré moyen d'attaque	Nombre de loges pourries par capsule
Glomerella				
I	0,7	1,4	0,8	1,5
II	0,6	1,1	0,7	1,3
III	0,7	1,3	0,8	1,4
IV	0,6	1,2	0,8	1,5
C. gossypii	1,1	2,4	1,8	3,3

d'une pourriture à *Fusarium* des cabosses de cacaoyer. L'étude réalisée par FORD, BOURRET et SNYDER (7) montre que :

1° - les souches présentant en culture la forme imparfaite (*Fusarium rigidiusculum*) (Brick) Snyd. et Hans) et la forme parfaite (*Calonectria rigidiuscula* (Berk. et Brme) Sacc.) ne sont pas pathogènes.

2° - Les souches pathogènes sont toutes des formes imparfaites.

3° - Certaines souches imparfaites pathogènes donnent lorsqu'on les confronte une ligne de périthèces.

Les deux premiers points sont conformes aux résultats de cette étude ; le troisième point n'a pas été étudié mais il est probable que, compte tenu de la sexualité particulière des *Glomerella*, la situation soit différente.

Ceci amène à la deuxième question posée par cette étude : le problème de la filiation *Glomerella - Colletotrichum gossypii* ; en effet, la première hypothèse pour tenter d'expliquer cette situation est que certaines formes *Glomerella* infectent à l'état latent le cotonnier et que, brusquement, pour une raison non déterminée une transformation fait apparaître à partir de ces formes un type pathogène déclenchant la maladie, la disparition de la forme sexuée pouvant être considérée comme une simplification de l'organisme liée à un parasitisme plus fort. Il est certain que dans le cas du *Fusarium* du cacaoyer une adaptation au parasitisme a fait disparaître la forme sexuée, la démonstration en est faite par le recouvrement de cette forme par croisement permettant par complémentation de reconstruire la chaîne des réactions métaboliques nécessaires à la formation du périthèce. En ce qui concerne *Glomerella*, une étude précédente (6) sur le déterminisme de la forme sexuée montre que les formes sauvages de *Glomerella* sont, *in vitro*, très instables pouvant évoluer vers une forme *Colletotrichum* sur le simple blocage d'un gène ; il est donc parfaitement possible que la forme imparfaite soit le résultat d'une évolution d'une forme parfaite vers la pathogénicité. Il n'a cependant pas été possible de rattacher ni morphologiquement ni sexuellement *Colletotrichum gossypii* à un type de *Glomerella* ; à ce dernier titre, toutes les souches de *Glomerella* ont d'ailleurs montré une individualité parfaite et il n'a pas été possible de réaliser des croisements entre formes + et — de souches strictement identiques morphologiquement et isolées par-

fois du même organe alors que ces croisements sont aisés entre mutants d'une même forme sauvage. D'autre part, si toutes les formes parfaites isolées sont dénuées de tout pouvoir pathogène, la réciproque n'est pas vrai et certaines souches imparfaites à petites spores ne sont pas non plus pathogènes, (ce type se rattache d'ailleurs morphologiquement aux formes *Glomerella* du groupe IV) ; enfin, les souches conidiennes mutantes de deux souches du groupe IV ne sont pas pour autant devenues pathogènes.

Tout ceci indique, d'une part, que s'il est probable qu'une adaptation puisse faire évoluer une forme parfaite vers une forme imparfaite, il s'agit plutôt d'une conséquence et il n'y a pas de liaison étroite entre le mode de reproduction et le pouvoir pathogène, d'autre part qu'une filiation n'est pas évidente et rien ne permet d'affirmer que *Colletotrichum gossypii* apparaisse par mutation d'une forme *Glomerella* décrite dans cette étude. *Glomerella cingulata* est un champignon courant dans les pays à climat chaud et humide, il est possible que ces formes soient d'origine locale et que *Colletotrichum gossypii* ait été introduit avec les premières graines de cotonnier.

Le problème des formes *Colletotrichum* à petites spores semblables morphologiquement à la forme imparfaite des *Glomerella* des groupes III et IV reste posé : s'agit-il d'une première évolution vers une virulence plus forte ? Et ces parasites peuvent-ils un jour fournir des pathogènes par mutation ou par échange parasexuel, entre eux ou avec la forme pathogène typique ?

CONCLUSION

Cette étude réalisée à l'origine dans le but d'étudire l'anthracnose du cotonnier à partir des formes parfaites de *C. gossypii* a finalement montré l'existence de différents types très voisins et l'absence de pouvoir pathogène des formes parfaites. Ces différences peuvent permettre de douter de la réalité de l'existence de l'espèce *Glomerella gossypii* Edg. qui a d'ailleurs été souvent critiquée. LEAKY et PERRY (11) après une étude taxonomique très complète concluent à la synonymie entre *G. cingulata* et *G. gossypii* d'après la description d'EDGERTON (3) et les premiers émettent des doutes sur l'existence d'une forme parfaite de *C. gossypii*. Par ailleurs, l'étude présente ne démontre pas une liaison physiologique entre aucune des formes *Glomerella* décrites et le cotonnier. On ne peut encore parler de formes spécialisées et celles-ci doivent être considérées comme différents types de *Glomerella cingulata* (Ston.) Sp. et Von Schr. dans le sens de VON ARX et MÜLLER (16).

Si les caractères taxonomiques ne permettent pas de maintenir *G. gossypii* comme une espèce différente de *G. cingulata*, l'étude d'EDGERTON ne permet pas non plus de conclure à l'existence d'une forme parfaite de *C. gossypii* ; celle-ci, en effet, ne mentionne pas de test de virulence avec des souches monospores et décrit ce stade présumé parfait de l'anthracnose sur *Gossypium herbaceum* et non sur *G. hirsutum* comme on pourrait s'y attendre. La même objection quant à l'absence de test de virulence peut être faite pour la dernière note signalant le stade parfait de *Colletotrichum gossypii* (10) ; la présence de périthèce de *Glomerella* sur une capsule mise à incuber suffit à annoncer l'existence de *Glomerella gossypii*. L'étude présente montre que si effectivement la présence de *Glomerella* est fréquente sur capsules, même sur capsules portant des lésions caractéristiques d'anthracnose, il est hâtif de conclure qu'il s'agit de la forme parfaite de *Colletotrichum gossypii* et les tests de virulence réalisés à partir de souches monospores montrent au contraire qu'il s'agit vraisemblablement de champignons différents. Si une description morphologique est nécessaire, elle est rarement suffisante et seule une étude du pouvoir pathogène peut décider de l'identité de deux organismes ; c'est un principe de base souvent peu respecté.

En définitive, le problème souvent posé de la synonymie entre *G. gossypii* Edg. et *G. cingulata* Ston. (Sp. et Schr.) n'est probablement pas à envisager car l'existence d'une forme *Glomerella* pathogène du cotonnier n'a jamais été réellement démontrée. Toutes les études concernant l'anthracnose du cotonnier font, par contre, état de l'utilisation de formes imparfaites, en particulier l'étude réalisée par ULLSTRUP (15) sur les « variations chez *Glomerella gossypii* » ne mentionne aucune forme parfaite ; c'est pourquoi nous pensons que, jusqu'à preuve du contraire, la dénomination *Colletotrichum gossypii* South. est la seule valable. Les similitudes existant entre cette forme et certaines formes de *Glomerella* parasites du cotonnier doivent cependant retenir l'attention et l'existence d'une forme sexuée peut permettre d'analyser certains phénomènes communs.

RÉSUMÉ

Quatre formes de *Glomerella* et deux formes de *Colletotrichum* (*C. gossypii* South. et *C.* à spores falciformes) isolées du cotonnier de nécroses de racines (*C. gossypii*), de nécroses de capsules (*C. gossypii*, *C.* à spores falciformes et *Glomerella* sp.) et d'infection latente (*C. gossypii* et *Glomerella* sp.) sont étudiées sur plantules et sur capsules pour leur pouvoir pathogène.

Seule la forme typique *Colletotrichum gossypii* South. est pathogène sur plantules et sur capsules quelque soit le mode d'infection.

Les formes *Glomerella* et la forme *Colletotrichum* à spores falciformes ne provoquent des pourritures sur capsules que si ces organismes sont introduits par piqûres.

Ces résultats sont analysées compte tenu d'études précédentes réalisées en particulier sur l'anthracnose du caféier *arabica*. Une filiation entre la forme typique *C. gossypii* et certains types de *Glomerella* très voisins morphologiquement n'est pas évidente ; la dénomination *Glomerella gossypii* Edg. peut faire l'objet de critique.

BIBLIOGRAPHIE

1. CAUQUIL J. et P. MILDNER, 1965. — Première étude sur le comportement variétal du cotonnier en présence des pourritures de capsules. *Cot. Fib. trop.*, 20, 4, 539-548.

2. COGNEE M. — Rapport de la Section de Phytopathologie de la Station de l'I.R.C.T. à Bouaké, 1960-61 et 1961-62.

3. EDGERTON C.W., 1909. — The perfect stage of the cotton anthracnose. *Mycologia*, 1, 114-120.

4. EDGERTON C.W., 1913. — Plus and minus strains in the genus *Glomerella*. *Amer. J. Bot.*, 1, 244-254.

5. FOLLIN J.C., 1969. — Sur les différentes formes de *Glomerella* et de *Colletotrichum* isolées du cotonnier. I. Localisation, étude morphologique. *Cot. Fib. trop.*, 24, 3.

6. FOLLIN J.C., 1968. — Sur le déterminisme de la formation du périthèce chez *Glomerella cingulata* f. sp. *gossypii*. *Cot. Fib. trop.*, 23, 4, 447-451.

7. FORD E.J., J.A. BOURRET et W.C. SNYDER, 1967. — Biologic specialization in *Calonectria* (*Fusarium*) *rigidiuscula* in relation to green point gall of Cocoa. *Phytopath.*, 57, 710-712.

8. HALISKY P.M., W.C. SCHNATHORST et M.A. SHAGRUN, 1961. — Severity and distribution of cotton bolls rots as related to temperature. *Phytopath.*, 51, 8, 501-505.

9. HOCKING D., 1966. — Brown blight (*Colletotrichum coffeanum* Noak) of arabica coffea in East Africa. *Ann. appl. Biol.*, 58, 409-421.

10. KURIAN N.J. et L. MOMIZ, 1966. — Perfect stage of *Colletotrichum gossypii* in Indian. *Indian Phytopath.*, 19, 4, 383-384.

11. LEAKY C.L.A. et D.A. PERRY, 1966. — The relation between damage caused by insect pests and boll rot associated with *Glomerella cingulata* (Ston.) Sp. et Schr. (*Colletotrichum gossypii* South.) on Upland Cotton in Uganda. *Ann. appl. Biol.*, 57, 337-344.

12. NUTSMAN F.J. et F.M. ROBERTS. — Investigations on a disease of *Coffea arabica* caused by a form of *Colletotrichum coffeanum* Noack.
I. Some factors affecting infection by the pathogen. *Trans. Brit. myc. Soc.*, 43, 3, 489-505. 1960.
II. The relation between infection of bearing wood and disease incidence. *Trans. Brit. myc. Soc.*, 44, 3, 511-521. 1961.

13. RAYNER R.W., 1948. — Latent infection in *Coffea arabica*. *Nature, Lond.*, 161, 245-246.

14. ROVIRA A.D., 1956. — Plant root excretions in relation to the rhizosphere effect. *Plt and Soil*, 7, 178-217.

15. ULLSTRUP A.J., 1938. — Variability of *Glomerella gossypii*. *Phytopath.*, 28, 787-798.

16. VON ARX J.A. et E. MULLER, 1954. — Die Gattungen der amerosporen Pyrenomyceten. *Breitr. Kryptoflora Schweiz*, 11, 1.

SUMMARY

Four Glomerella *forms and two* Colletotrichum *forms* (C. gossypii *South. and* C. *with falciform spores*) *isolated from cotton, from root necroses* (C. gossypii), *boll necroses* (C. gossypii, C. *with falciform spores and* Glomerella *sp.*) *and from latent infection* (C. gossypii *and* Glomerella *sp.*) *are studied on seedlings and on bolls in relevance with their pathogenic power.*

Only the Colletotrichum gossypii *South. typical form is pathogenic on seedlings and on bolls whatever the mode of infection.*

The Glomerella *forms and the* Colletotrichum *form with falciform spores induce boll rot only if these organisms are introduced by punctures.*

These results are analysed taking in consideration earlier studies pursued in particular on anthracnose of Arabica *coffee; a filiation between the* C. gossypii *typical form and some* Glomerella *types very closely related morphologically is not evident. The denomination of* Glomerella gossypii *Edg. may form the object of criticism.*

RESUMEN

Cuatro formas de Glomerella *y dos formas de* Colletotrichum (C. gossypii *South. y* C. *a esporas falciformes*) *aislados del algodonero de necrosis de raíces* (C. gossypii), *de necrosis de cápsulas* (C. gossypii, C. *a esporas falciformes y* Glomerella *sp.*) *y de infección latente* (C. gossypii *y* Glomerella *sp.*) *se estudian sobre plántulas y sobre cápsulas por su poder patógeno.*

Solo la forma típica Colletotrichum gossypii *South. es patógena sobre plántulas y sobre cápsulas cualquiera que sea el modo de infección.*

Las formas Glomerella *y la forma* Colletotrichum *a esporas falciformes no provocan podredumbres en las cápsulas a no ser que esos organismos sean introducidos por inyecciones.*

Estos resultados se analizan teniendo en cuenta estudios precedentes realizados en particular sobre la antracnosis del cafeto arabica; una filiación entre la forma típica C. gossypii *y ciertos tipos de* Glomerella *muy próximos morfológicamente no es evidente, la denominación* Glomerella gossypii *puede ser objeto de crítica.*

ORIGINE ET MISE EN PLACE DES FIBRES DANS LES PARTIES AÉRIENNES DE L'*Hibiscus cannabinus* (Malvacées) (1)

par

J. BOURÉLY

NOTE DE L'AUTEUR.

*— Une connaissance plus précise de l'origine et de la mise en place des fibres textiles dans les parties aériennes de l'*Hibiscus cannabinus *nous a paru nécessaire avant d'entreprendre, dans le cadre même des Etudes menées à l'I.R.C.T., des travaux de portée plus directement pratique et susceptibles, en particulier, d'être exploités dans les différents secteurs d'amélioration de la production des fibres (agronomie, génétique, technologie).*

— Cette étude a été réalisée au Laboratoire de Madame NOUGARÈDE *- Professeur de Botanique - Laboratoire de Cytologie et Morphogenèse Végétales, 9, quai Saint-Bernard - 75 - Paris-5e*

L'*Hibiscus cannabinus* L. (Malvacées) est une plante textile tropicale dont la tige au port dressé (2,5 à 3 m de hauteur) fournit une fibre utilisable en sacherie en remplacement de celle du Jute. Nous nous proposons, dans cette étude, de déterminer l'origine et la mise en place des différents types de fibres, dans l'hypocotyle et l'épicotyle de la variété « I.R.C.T.. Soudan Précoce ». Pour réaliser ce travail, nous suivrons les modalités de différenciation des tissus depuis la graine jusqu'à l'édification de la dix-septième feuille produite par le point végétatif (50 cm de haut). Nous utiliserons les techniques anatomiques classiques et essentiellement des sections transversales pratiquées à divers niveaux.

Développement des fibres dans l'hypocotyle

Les figures 1 et 2 donnent l'aspect morphologique de germinations depuis 24 h jusqu'à 144 h. Dès 24 h de germination, alors que la plantule est encore incluse dans la graine (fig. 1 a), les premiers éléments de xylème (fig. 3, xy) et de phloème (fig. 3, ph) se différencient dans l'hypocotyle à 200 μ du point végétatif. Après 48 h, (fig. 4 a et b), on distingue, à la base de l'hypocotyle, au niveau marqué, ab, sur la figure 1b, quatre ou parfois cinq massifs de xylème primaire, xy, qui alternent avec ceux du phloème primaire, ph. Après 200 h de germination, ces derniers (fig. 5, ph) sont eux-mêmes segmentés par des cellules de parenchyme, pl, en cinq ou six « sous-massifs » phloémiens, de six à douze cellules chacun, en section transversale. A l'intérieur d'un même massif de phloème ainsi subdivisé en sous-massifs (soulignés par les flèches épaisses), ce sont les sous-massifs les plus internes dont les cellule épaississent et lignifient leurs parois (fig. 5, flèches fines); les cellules des sous-massifs les plus externes, situées près du xylème, demeurent plus longtemps non épaissies.

Ainsi, les phénomènes d'épaississement progressent depuis les sous-massifs situés au centre du massif phloémien jusqu'aux sous-massifs proches du xylème. Dès ce stade (fig. 5), un cambium, c, s'est déjà formé. Après 220 h de germination (fig. 6 a), un très grand nombre de cellules de phloème se sont déjà transformées en fibres lignifiées, fp, dans chaque sous-massif. *Ces fibres d'origine primaire et phloémienne sont les premières formées.* Elles sont responsables de l'extrême dureté de l'hypocotyle. Ultérieurement (fig. 6 b), toutes les cellules des massifs de phloème à l'exception du parenchyme, pl, épaissiront leurs parois. Le cambium mis en place au tout début de la germination (150 h) produit en direction de

(1) Note présentée à l'Académie des Sciences de Paris et parue dans les *C.R. Acad. Sc. Paris*, Série D, t. 268, p. 1714-1717 (31 mars 1969).

de plus de 20 jours ne sont pratiquement plus attaquées par les chenilles de la capsule.

Le gossypol ne peut donc pas jouer un rôle dans l'attaque des capsules par les chenilles. Le niveau plus bas du taux de gossypol dans les capsules âgées des cotonniers sans glandes est par conséquent sans importance.

CONCLUSION

Les élevages sur milieu artificiel sont en général relativement faciles à réaliser. Par conséquent, il existe une tendance de chercher de plus en plus la solution des problèmes entomologiques dans des essais réalisés en laboratoire. Les résultats que nous venons de donner suggèrent que ces essais fournissent quelquefois des résultats d'une application restreinte.

BOTTGER et PATANA (1966) ont, par exemple, déterminé les DL 90 de gossypol vis-à-vis de quelques insectes nuisibles du cotonnier ; ils trouvent une toxicité relativement él
les auteurs suggèr
sauvages avec un
croiser avec les co
menter le taux du
des insectes nuisib

L'efficacité d'une
teuse dans le cas
fructifères.

Nos résultats su
sans glandes, d'au
gossypol ont subi
résultat une réduc
et par *H. armigera*
par *Earias* spp.

L'étude détaillée
être éclaircir les
cotonniers.

1969

FOLLIN, J.C.

Sur les différentes formes de Glomerella Spaul. et Schr. et de Colletotrichum Cda isolées du Cotonnier. I. Localisation et étude morphologique. II. Etude du pouvoir pathogène, premières conclusions. [On the different forms of Glomerella and Colletotrichum isolated from Cotton. I. Localization and morphological study. II. Pathogenicity study, first conclusions.]

Coton Fibr. trop., 24 (3):337-343; 345-350. [1 col. pl. (2 fig.), 1 fig., 6 tables; 1 pl. (5 fig.), 1 graph, 4 tables. 15 ref. Engl. P.T.O.

RESUME

En général, la littérature signale une grande sensibilité des cotonniers sans glandes vis-à-vis de plusieurs insectes, notamment *Heliothis zea*.

Des recherches effectuées pendant trois ans au Tchad ont révélé que les cotonniers sans glandes subissent des attaques plus fortes d'*Earias* spp., mais sont moins attaqués par les chenilles d'*Heliothis armigera* et de *Diparopsis watersi*. Il se trouve que l'absence du gossypol ne peut jouer un rôle dans l'attaque des capsules des cotonniers sans glandes par les chenilles de la capsule.

BIBLIOGRAPHIE

BOTTGER G.T. et R. PATANA, 1966. — Growth, development and survival of certain Lepidoptera fed gossypol in the diet. *J. econ. Ent.*, 59, 1166-1168.

BOTTGER G.T., E.T. SHEEHAN et M.J. LUKEFAHR, 1964. — Relation of gossypol content of cotton plants to insect resistance. *J. econ. Ent.*, 57, 283-285.

BRADER L., 1967. — La faune des cotonniers sans glandes dans la partie méridionale du Tchad - I - Altises. *Cot. Fib. trop.*, 22, 171-181.

GALLUP W.D., 1928. — A chemical study of the development of cotton bolls and the rate of formation of gossypol in the cotton seed. *J. agr. Res.*, 36, 471-480.

JENKINS J.N., F.G. MAXWELL et H.N. LAFEVER, 1966. — The comparative preference of insects for glanded and glandless cottons. *J. econ. Ent.*, 59, 352-356.

LUKEFAHR M.J., L.W. NOBLE et J.E. HOUGHTALING, 1966. — Growth and infestation of bollworms and other insects on glanded and glandless strains of cotton. *J. econ. Ent.*, 59, 817-820.

MAXWELL F.G., J.N. JENKINS, H.N. LAFEVER, M.J. LUKEFAHR et G.T. BOTTGER, 1965. — Insect situation on glandless cotton. A progress report. *Nat. Cott. Counc., Proc. 17th ann. Cott. Improv. Conf.*, Atlanta, Georgia, 73-74.

REEVES R.G. et J.O. BEASLEY, 1935. — The development
5-944.

ics aspects
r buds of

mistry and
plant, with
ds in rela-

cotton is
insects, in

hree years
nore seve
y attacked
si worms.
It happens that the absence of gossypol cannot have any part in the attack of glandless cotton by boll worms.

RESUMEN

En general, la literatura señala una gran sensibilidad de los algodoneros sin glándulas con respecto a varios insectos, principalmente Heliothis zea.

Investigaciones efectuadas durante tres años en el Tchad, han puesto de manifiesto que los algodoneros sin glandulas experimentan ataques más fuertes del Earias *spp. pero son menos atacados por las orugas del* Heliothis armigera *y del* Diparopsis watersi. *Ocurre que la ausencia del gossypol no puede desempeñar ningún papel en el ataque de las cápsulas de los algodoneros sin glándulas por las orugas de la cápsula.*

SUR LES DIFFÉRENTES FORMES DE *Glomerella* Spaul. et Schr. ET DE *Colletotrichum* Cda ISOLÉES DU COTONNIER

I. - Localisation et étude morphologique

par

J. C. FOLLIN

Phytopathologiste
Station Centrale de BAMBARI

Ce travail a été réalisé en 1967 et 1968 sur la Station de BAMBARI et au Laboratoire de Biologie expérimentale de la Faculté des Sciences d'ORSAY (Service de Cryptogamie).

INTRODUCTION

L'isolement, au cours de travaux de routine, de souches de *Colletotrichum* très diverses dans leur morphologie a conduit à réaliser des isolements systématiques dans les différents organes du cotonnier à différents stades et à constituer une collection des différents types rencontrés.

Le but de ce travail était triple : tout d'abord voir s'il existe une spécialisation de certains types de souches, c'est-à-dire si la répartition est différente suivant les organes, ensuite définir l'évolution d'une population de *Glomerella* et de *Colletotrichum* en fonction de la croissance du cotonnier, enfin étudier la virulence des différentes formes en vue d'une étude génétique éventuelle du pouvoir pathogène à l'aide de formes à reproduction sexuée.

L'étude présente concerne les deux premiers points.

MATÉRIEL ET MÉTHODES

Les isolements ont été réalisés à partir de graines, de plantules, de tiges et de capsules des variétés D 9, B 50, et BJA 592 (*Gossypium hirsutum L.*). Les techniques sont les suivantes :

1. *Graines.* Les graines sont délintées à l'acide sulfurique, désinfectées au bichlorure de mercure à 0,1 % (10 mn) et mises en tubes sur culot de PDA ; ces isolements sont faits sur graines entières ou décortiquées.

2. *Plantules*

— Plantules malades : les parties atteintes sont désinfectées deux minutes au bichlorure de mercure à 0,1 % et mises en boite de Pétri sur eau gélosée ; les colonies sont ensuite repiquées sur PDA.

— Plantules saines : les plantules sont divisées en 3 parties, racines, tiges et feuilles ; ces organes sont désinfectés soit au xylol (bain rapide) et au bichlorure de mercure (2 mn), soit au bichlorure seul et mis sur eau gélosée comme précédemment (fig. 1).

3. *Tiges.* Les tiges non décortiquées sont désinfectées au xylol (bain rapide), alcool, bichlorure de mercure (10 mn), chlorure de chaux (20 mn) et mises sur gélose en boite de Pétri ; les tiges décortiquées et les pétioles sont seulement désinfectés au bichlorure de mercure (10 et 3 mn).

4. *Capsules.* Les isolements sont réalisés à partir de capsules momifiées, de capsules portant des lésions typiques et de capsules saines ; une partie de ces derniers isolements concerne une étude réalisée par J. CAUQUIL (résultats non publiés) sur l'infection latente du cotonnier ; ils sont faits à partir de rondelles de péricarpe, du sommet de la capsule et de l'axe central ; dans tous les cas, le milieu d'isolement est l'eau gélosée.

Les souches sont identifiées sur bouillon de pomme de terre glucosé et gélosé (PDA Difco), sur décoction de haricot de Lima gélosé (Lima bean Agar Difco) et sur bouillon d'avoine gélosé.

Les thalles sont cultivés à l'étuve à 26 °C ou à la température du laboratoire (comprise entre 23 et 30 °C).

Trois séries d'isolement ont été réalisés :

A. Isolements dans différents organes du cotonnier pris au hasard dans différents endroits, ceci dans le but de constituer une collection et d'étudier l'existence éventuelle d'une répartition suivant les organes.

B. Comparaison d'isolements réalisés sur plantules portées par un champ en ouverture et par un champ en quatrième année de culture de coton, les deux parcelles d'observations étant contiguës.

C. Evolution d'une population parallèlement à l'évolution de la plante : isolement sur feuilles cotylédonnaires, feuilles, tiges décortiquées, non décortiquées et sur capsules.

de plus de 20 jours ne sont pratiquement plus attaquées par les chenilles de la capsule.

Le gossypol ne peut donc pas jouer un rôle dans l'attaque des capsules par les chenilles. Le niveau plus bas du taux de gossypol dans les capsules âgées des cotonniers sans glandes est par conséquent sans importance.

CONCLUSION

Les élevages sur milieu artificiel sont en général relativement faciles à réaliser. Par conséquent, il existe une tendance de chercher de plus en plus la solution des problèmes entomologiques dans des essais réalisés en laboratoire. Les résultats que nous venons de donner suggèrent que ces essais fournissent quelquefois des résultats d'une application restreinte.

BOTTGER et PATANA (1966) ont, par exemple, déterminé les DL 90 de gossypol vis-à-vis de quelques insectes nuisibles du cotonnier ; ils trouvent une toxicité relativement élevée. Sur la base de ces données, les auteurs suggèrent de rechercher des cotonniers sauvages avec un taux de gossypol élevé et de les croiser avec
menter le t
des insectes

L'efficacit
teuse dans
fructifères.

Nos résu
sans glande
gossypol on
résultat un
et par *H. ar*
par *Earias*

L'étude d
être éclairc
cotonniers.

Span. summ. 7

Microfilm no.

15620

En général, la littérature signale une grande sensibilité des cotonniers sans glandes vis-à-vis de plusieurs insectes, notamment *Heliothis zea.*

Des recherches effectuées pendant trois ans au Tchad ont révélé que les cotonniers sans glandes subissent des attaques plus fortes d'*Earias* spp., mais sont moins attaqués par les chenilles d'*Heliothis armigera* et de *Diparopsis watersi.* Il se trouve que l'absence du gossypol ne peut jouer un rôle dans l'attaque des capsules des cotonniers sans glandes par les chenilles de la capsule.

BIBLIOGRAPHIE

BOTTGER G.T. et R. PATANA, 1966. — Growth, development and survival of certain Lepidoptera fed gossypol in the diet. *J. econ. Ent.*, 59, 1166-1168.

BOTTGER G.T., E.T. SHEEHAN et M.J. LUKEFAHR, 1964. — Relation of gossypol content of cotton plants to insect resistance. *J. econ. Ent.*, 57, 283-285.

BRADER L., 1967. — La faune des cotonniers sans glandes dans la partie méridionale du Tchad - I - Altises. *Cot. Fib. trop.*, 22, 171-181.

GALLUP W.D., 1928. — A chemical study of the development of cotton bolls and the rate of formation of gossypol in the cotton seed. *J. agr. Res.*, 36, 471-480.

JENKINS J.N., F.G. MAXWELL et H.N. LAFEVER, 1966. — The comparative preference of insects for glanded and glandless cottons. *J. econ. Ent.*, 59, 352-356.

LUKEFAHR M.J., L.W. NOBLE et J.E. HOUGHTALING, 1966. — Growth and infestation of bollworms and other insects on glanded and glandless strains of cotton. *J. econ. Ent.*, 59, 817-820.

MAXWELL F.G., J.N. JENKINS, H.N. LAFEVER, M.J. LUKEFAHR et G.T. BOTTGER, 1965. — Insect situation on glandless cotton. A progress report. *Nat. Cott. Counc., Proc. 17th ann. Cott. Improv. Conf.*, Atlanta, Georgia, 73-74.

REEVES R.G. et J.O. BEASLEY, 1935. — The development of the cotton embryo. *J. agr. Res.*, 51, 935-944.

RHYNE C.L. et F.H. SMITH, 1965. — Genetics aspects
nd flower buds of
.

. — Chemistry and
cotton plant, with
lar glands in rela-
3.

ndless cotton is
everal insects, in

d for three years
s are more seve
severely attacked
s watersi worms.
ypol cannot have
s cotton by boll

RESUMEN

En general, la literatura señala una gran sensibilidad de los algodoneros sin glándulas con respecto a varios insectos, principalmente Heliothis zea.

Investigaciones efectuadas durante tres años en el Tchad, han puesto de manifiesto que los algodoneros sin glandulas experimentan ataques más fuertes del Earias *spp. pero son menos atacados por las orugas del* Heliothis armigera *y del* Diparopsis watersi. *Ocurre que la ausencia del gossypol no puede desempeñar ningún papel en el ataque de las cápsulas de los algodoneros sin glándulas por las orugas de la cápsula.*

SUR LES DIFFÉRENTES FORMES DE *Glomerella* Spaul. et Schr. ET DE *Colletotrichum* Cda ISOLÉES DU COTONNIER

I. - Localisation et étude morphologique

par

J. C. FOLLIN

Phytopathologiste

Station Centrale de BAMBARI

Ce travail a été réalisé en 1967 et 1968 sur la Station de BAMBARI et au Laboratoire de Biologie expérimentale de la Faculté des Sciences d'ORSAY (Service de Cryptogamie).

INTRODUCTION

L'isolement, au cours de travaux de routine, de souches de *Colletotrichum* très diverses dans leur morphologie a conduit à réaliser des isolements systématiques dans les différents organes du cotonnier à différents stades et à constituer une collection des différents types rencontrés.

Le but de ce travail était triple : tout d'abord voir s'il existe une spécialisation de certains types de souches, c'est-à-dire si la répartition est différente suivant les organes, ensuite définir l'évolution d'une population de *Glomerella* et de *Colletotrichum* en fonction de la croisance du cotonnier, enfin étudier la virulence des différentes formes en vue d'une étude génétique éventuelle du pouvoir pathogène à l'aide de formes à reproduction sexuée.

L'étude présente concerne les deux premiers points.

MATÉRIEL ET MÉTHODES

Les isolements ont été réalisés à partir de graines, de plantules, de tiges et de capsules des variétés D 9, B 50, et BJA 592 (*Gossypium hirsutum L.*). Les techniques sont les suivantes :

1. *Graines.* Les graines sont délintées à l'acide sulfurique, désinfectées au bichlorure de mercure à 0,1 % (10 mn) et mises en tubes sur culot de PDA ; ces isolements sont faits sur graines entières ou décortiquées.

2. *Plantules*

— Plantules malades : les parties atteintes sont désinfectées deux minutes au bichlorure de mercure à 0,1 % et mises en boîte de Pétri sur eau gélosée ; les colonies sont ensuite repiquées sur PDA.

— Plantules saines : les plantules sont divisées en 3 parties, racines, tiges et feuilles ; ces organes sont désinfectés soit au xylol (bain rapide) et au bichlorure de mercure (2 mn), soit au bichlorure seul et mis sur eau gélosée comme précédemment (fig. 1).

3. *Tiges.* Les tiges non décortiquées sont désinfectées au xylol (bain rapide), alcool, bichlorure de mercure (10 mn), chlorure de chaux (20 mn) et mises sur gélose en boîte de Pétri ; les tiges décortiquées et les pétioles sont seulement désinfectés au bichlorure de mercure (10 et 3 mn).

4. *Capsules.* Les isolements sont réalisés à partir de capsules momifiées, de capsules portant des lésions typiques et de capsules saines ; une partie de ces derniers isolements concerne une étude réalisée par J. CAUQUIL (résultats non publiés) sur l'infection latente du cotonnier ; ils sont faits à partir de rondelles de péricarpe, du sommet de la capsule et de l'axe central ; dans tous les cas, le milieu d'isolement est l'eau gélosée.

Les souches sont identifiées sur bouillon de pomme de terre glucosé et gélosé (PDA Difco), sur décoction de haricot de Lima gélosé (Lima bean Agar Difco) et sur bouillon d'avoine gélosé.

Les thalles sont cultivés à l'étuve à 26 °C ou à la température du laboratoire (comprise entre 23 et 30 °C).

Trois séries d'isolement ont été réalisés :

A. Isolements dans différents organes du cotonnier pris au hasard dans différents endroits, ceci dans le but de constituer une collection et d'étudier l'existence éventuelle d'une répartition suivant les organes.

B. Comparaison d'isolements réalisés sur plantules portées par un champ en ouverture et par un champ en quatrième année de culture de coton, les deux parcelles d'observations étant contiguës.

C. Evolution d'une population parallèlement à l'évolution de la plante : isolement sur feuilles cotyledonnaires, feuilles, tiges décortiquées, non décortiquées et sur capsules.

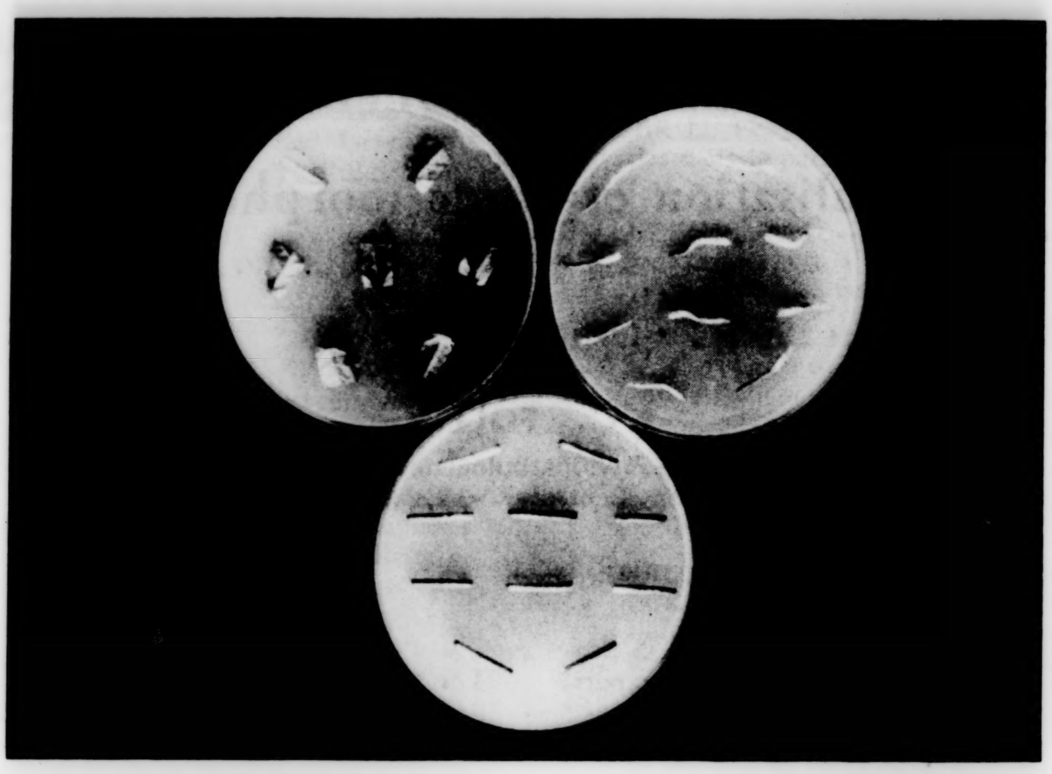


Fig. 1. — Mode d'isolement sur gélose des différentes parties d'une plantule, la technique est identique pour les capsules vertes (rondelles de péricarpe et sommets de capsules).

RÉSULTATS

Etude morphologique

Cette étude a un but pratique et non systématique ; elle reste sommaire et a été réalisée afin de dégager, par quelques traits essentiels (couleur du mycélium, mode de reproduction asexué, mensuration des spores), les groupes principaux de *Glomerella* et *Colletotrichum* parasites du cotonnier. Le classement n'est d'ailleurs pas d'une rigueur absolue et certaines souches hors types n'ont pu être classées avec certitude, le mode de reproduction asexuée est le point qui, dans les cas douteux, a décidé de la répartition.

Les caractéristiques des différentes formes sont consignées dans les tableaux I et II et illustrées par les figures 2 et 3.

En première approximation, on peut distinguer deux grandes catégories d'isolements : ceux qui ne conduisent qu'au stade *Colletotrichum* et ceux qui produisent en plus des périthèces. Cette dernière catégorie comporte deux groupes principaux : les formes *Glomerella* avec peu ou pas de conidies et les formes en produisant abondamment.

Dans la pratique, le processus de classement est le suivant :

1. Repiquage sur PDA et LBA ; les tubes sont laissés à la lumière naturelle ou artificielle, ceci permet de reconnaître les groupes I et II ; il reste les souches produisant de nombreux acervules sur LBA.

2. Ces souches restantes sont repiquées sur avoine et mises à l'obscurité, ce qui permet de distinguer les groupes III et IV.

La forme *Colletotrichum* à spores falciformes n'a pas reçu de nom d'espèce ; ses mensurations peuvent la faire considérer comme une race de *C. indicum* Dast., mais nous l'avons également observée sur Poivron (*C. annuum*) et sur Gombo (*H. esculentus*) ; il peut s'agir tout aussi bien de *C. capsici* (Syd.) Butl. et Bir.

Répartition suivant les organes

Dans tous les cas, un témoin constitué par un échantillon des graines semées au champ est mis à germer sur banc de sable stérile de façon à déterminer l'infection primaire. La forme *C. gossypii* est seule isolée et provoque généralement la mort de la plantule ; cette forme est certainement la seule à infecter les graines à l'intérieur.

Le tableau III donne les résultats principaux. Les séries C, D et H ont été réalisées sur plantules d'un champ portant des cotonniers pour la troisième année consécutive. Les organes sont désinfectés de deux manières : au bichlorure de mercure (série C et H), au xylol, puis au bichlorure de mercure (série D).

Sur plantules nécrosées et dans les graines, on ne trouve que la forme imparfaite typique *C. gossypii* South., sur lésions de capsules et sur capsules momifiées, par contre, les deux formes sont trouvées, la forme *Glomerella* plus fréquemment que la forme imparfaite comprenant *C. gossypii* et la forme *Colletotrichum* à spores falciformes.

PLANCHE I

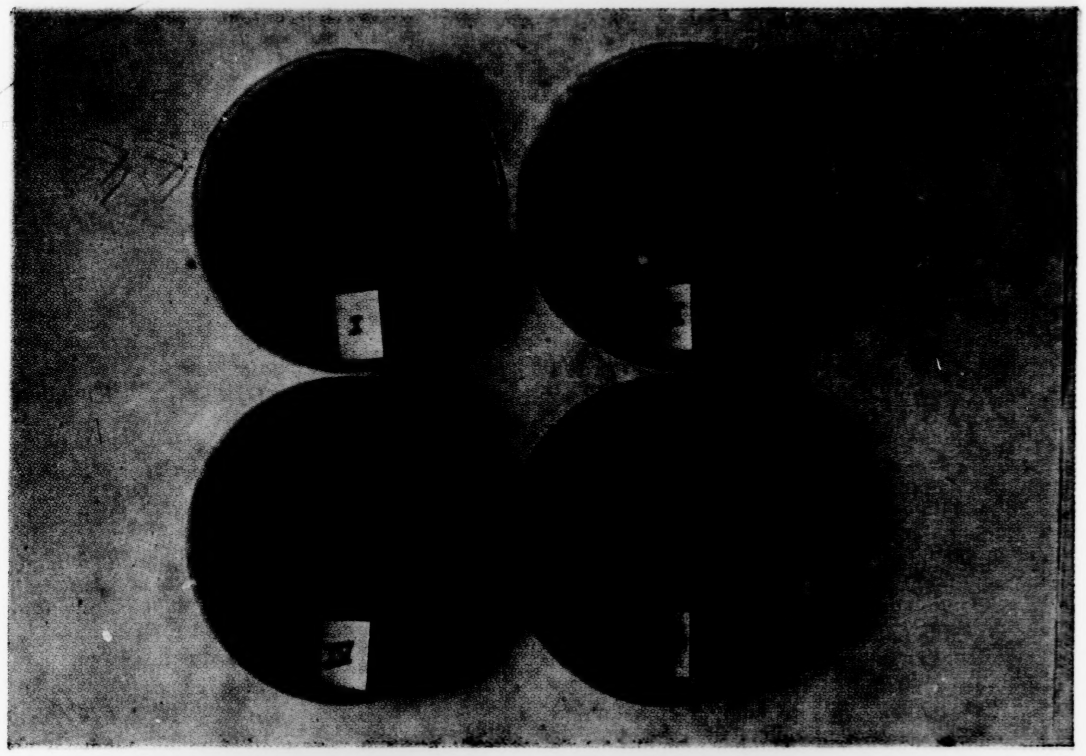


Fig. 2. — Aspect des quatre formes de *Glomerella* sp. sur milieu P.D.A.

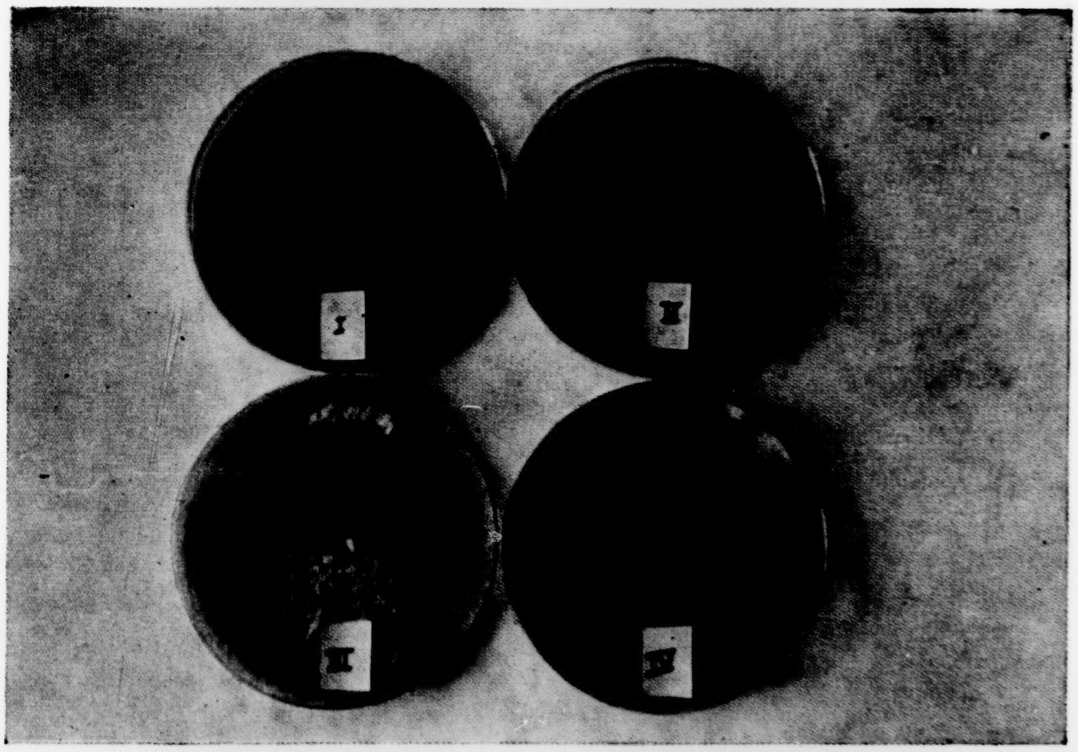


Fig. 3. — Aspect des quatre formes de *Glomerella* sp. sur milieu avoine.

Tableau I. — *Caractéristiques essentielles des différentes formes de* Glomerella *isolées du cotonnier et de* Colletotrichum gossypii *South.*

	Mycélium	Forme imparfaite	Forme parfaite	Forme des spores
Glomerella Groupe I	PDA : Myc. aérien gris acier, parfois reflet orangé. LBA : Myc. ras, blanc terne. Avoine : Myc. ras, gris pâle.	Conidies rares, cependant présence d'acervules avec des soies bien différenciées (3 à 4 par acervule).	PDA : périthèces en glomérules. LBA : périthèces en glomérules. Avoine : per. en glomérules et par groupe de 4 à 5.	Conidies cylindriques arrondies aux deux extrémités. I présente parfois des conidies apiculées.
Groupe II	PDA : Myc. aérien blanc à jaune milieu ocre jaune à orange. LBA : Myc. ras blanc terne. Avoine : Myc. ras gris pâle.	Identique à I.	PDA : identique à I. LBA : glomérules plus abondants que I. Avoine : identique à I.	Ascospores arquées en forme de croissant. Les spores de IV, à largeur plus faible sont de forme plus élancée.
Groupe III	PDA : Myc. aérien vert clair puis foncé. LBA : myc. ras blanc. Avoine : myc. blanc couvert d'acervules.	Sporulation abondante à la lumière et à l'obscurité. Acervules sans soies.	PDA : per. en glomérules. LBA : glomérules et per. isolés. Avoine : glomérules rares.	
Groupe IV	PDA : myc. aérien vert foncé. LBA : myc . ras blanc terne. Avoine : myc. ras gris acier.	Acervules sans soies et conidies abondantes dans les cultures exposées à la lumière.	PDA : per. en glomérules. LBA et avoine : glomérules à l'obscurité, glomérules et perithèces isolés à la lumière.	
Colletotrichum gossypii	PDA : myc. aérien vert foncé à noir. LBA : myc. ras blanc. Avoine : myc. ras, gris pâle à gris acier.	Sporulation abondante surtout sur LBA et avoine, présence de soies irrégulières sur LBA.		Conidies ovoïdes à piriformes arrondies au bout, droites, souvent apiculées à la base et étranglées au milieu.

Tableau II. — *Dimensions en microns des spores de* Glomerella *et* Colletotrichum *isolés de cotonniers (le milieu de culture est constitué par une décoction gélosée de flocons d'avoine).*

	Conidies	Ascospores
Glomerella I	13,0 × 3,5 (9,0-15,0) × (3,0-4,5)	15,9 × 4,2 (14,4-19,2) × (3,6-4,8)
II	—	15,1 × 4,0 (13,8-17,4) × (3,6-4,8)
III	12,1 × 3,5 (10,2-15,4) × (3,0-4,0)	15,1 × 3,6 (13,2-17,6) × (2,2-4,2)
IV	11,5 × 3,3 (10,2-14,4) × (3,0-3,6)	15,2 × 3,5 (13,2-18,0) × (2,4-4,2)
Colletotrichum gossypii	14,4 × 3,1 12,0-19,8) × (2,4-4,2)	—
Colletotrichum sp.	12,6 × 3,2 (10,2-15,0) × (3,0-3,6)	—
Colletotrichum à spores falciformes	23,0 × 2,9 (26-20,4) - (2,4-3,0)	—

Tableau III. — *Répartition des différentes formes de* Glomerella *et de* Colletotrichum *en fonction du lieu d'isolement.*

Lieux d'isolement	Nombre d'isolements	Forme imparfaite	Forme parfaite et f. p. + f. imp.
GRAINES			
décortiquées	60	1	—
non décortiquées	60	3	—
PLANTULES À RACINES NÉCROSÉES (lésions typiques d'anthracnose)	19	19	—
PLANTULES SAINES			
Série C :			
racines	130	5	2
tiges	130	3	—
feuilles	130	5	20
Série D :			
racines	130	1	—
tiges	130	2	—
feuilles	130	1	—
Série H : feuilles	130	6	15
TIGES - Infection latente	400	—	4
CAPSULES			
Série L :			
lésions typiques d'anthracnose	14	4	10
Série M :			
capsules momifiées	30	3	5
Série N :			
infection latente de la capsule	100	1	35

En infection interne, c'est la forme parfaite qui est le plus souvent isolée ; rare dans les tiges, elle est particulièrement abondante dans les feuilles et les téguments des capsules.

Importance suivant le précédent cultural (tableau IV)

Deux séries d'isolements sur cotonniers portés par un champ en ouverture montrent l'augmentation de la population de *Glomerella* dans le sol ; celle-ci passe de 36 %, par rapport au nombre de champignons isolés, à 78,6 % dans le champ en quatrième année de culture. Par contre, la situation des formes *Colletotrichum* n'évolue pas.

Evolution dans la plante (tableau V)

Ces résultats confirment les précédents. La population de *Glomerella* est particulièrement abondante en infection interne des capsules et des feuilles ; elle est plus rare dans les tiges et limitée aux parties non encore lignifiées. Le vieillissement des tissus favorise d'autres champignons, en particulier des *Phoma* et un *Phomopsis* sp. très fréquemment isolé.

Dans les parties non encore lignifiées, l'infection reste superficielle comme le montrent les isolements réalisés à partir de pétioles.

Tableau IV. — *Fréquence d'isolement des formes* Glomerella *et* Colletotrichum *de plantules (feuilles cotylédonnaires) portées par un champ en ouverture et par un champ cultivé en coton pour la quatrième année consécutive.*

	Champ en ouverture	Champ en 4ᵉ année
Nombre d'isolements	170	170
Nombre d'isolements positifs	38	61
Nombre de *Glomerella* et de *Colletotrichum*	14	48
Groupe I	6	17
Groupe II	0	12
Groupe III	2	11
Groupe IV	5	6
forme imparfaite typique	1	2
% *Glomerella* par rapport au nombre d'isolements	8,2	28,2
% *Glomerella* par rapport au nombre d'isolements positifs	36,8	78,6

Tableau V. — *Répartition des différentes formes parfaites et imparfaites en fonction de l'âge du cotonnier.*

Lieux d'isolement	Nombre d'isolements réalisés	Nombre d'isolements positifs	Forme parfaite et f.p. + f. imparfaite	F. imparfaite
Plants de 10 jours				
Feuilles cotylédonnaires	170	61	46	2
Plants de 4 semaines				
Derniers entrenœuds	60	9	2	—
Dernières feuilles (6ᵉ)	125	12	3	—
Plants de 2 mois				
Feuilles vers le sommet	90	50	41	—
Pétioles	90	13	7	—
Plants de 2,5 mois				
Bas de tiges	45	43	1	—
Bas de tiges décortiquées	45	8	—	—
Avant-derniers entrenœuds	45	19	7	—
Plants de 3 mois				
Capsules				
• Sommets	100	48	29	4*
• Pédoncules	50	38	21	—
• Péd. décortiqués	50	16	—	—

* 1 souche à spores falciformes.

Tableau VI. — *Répartition dans les groupes I, II, III et IV des différentes souches de* Glomerella *observées.*

Lieux d'isolement	Nombre de souches observées	Groupe I %	Groupe II %	Groupe III %	Groupe IV %
Feuilles cotylédonnaires	72	47,2	23,6	16,6	12,6
Feuilles	40	27,5	62,5	5,0	5,0
Tiges non aoutées et pétioles de feuilles	14	35,7	42,8	—	21,5
Capsules :					
Sommets	63	30,3	23,8	19,0	26,9
Pétioles	21	19,0	62,0	—	19,0
Capsules momifiées et capsules avec lésions externes	15	26,6	—	46,8	26,6

Les formes *Colletotrichum* sont isolées beaucoup plus rarement et seulement de plantules et de capsules.

Répartition des différentes formes de *Glomerella* (tableau VI)

En infection interne, ce sont les formes I et II qui sont le plus fréquemment isolées ; par contre, la situation est inversée lorsqu'il s'agit d'isolements à partir de capsules lésées ou de capsules momifiées, ce sont alors les formes possédant la forme imparfaite et sporulant abondamment (groupes III et IV) qui prédominent.

DISCUSSION

Une première constatation est qu'il n'y a pas de répartition particulière suivant les organes : des formes parfaites et imparfaites sont isolées de racines, de tiges et de capsules ; par contre, on peut constater une spécialisation particulière ; en effet, dans les cas de lésions typiques sur plantules et à l'intérieur des graines, on isole toujours la forme imparfaite typique de l'anthracnose, de même que dans le cas d'isolement sur plantules désinfectées très sévèrement par un bain dans le xylol qui détruit les organismes d'une façon plus profonde que le traitement au bichlorure de mercure. Il semble

donc pour les plantules que *C. gossypii* soit spécifique des nécroses et des infections internes profondes, infection interne ou début d'attaque encore invisible à l'œil nu.

Des plantules désinfectées au bichlorure de mercure, on isole toutes les formes, les formes *Glomerella* correspondraient à une infection interne superficielle. Des plants plus âgés, on n'isole ces formes que des parties non encore lignifiées, et il est probable non seulement qu'elles sont incapables de franchir le cambium lorsque la structure est secondaire, mais qu'elles restent également superficielles dans les parties vertes comme le montrent les isolements à partir de pétioles.

Dans le cas des capsules, la situation est différente. Il est en effet très courant d'observer la forme parfaite sur des lésions typiques d'anthracnose, ce qui ne démontre d'ailleurs pas le caractère de parasite primaire, les capsules pouvant être soumises à des déprédations multiples (chenilles, piqûres d'Hémiptères...). Par ailleurs, le grand nombre de formes *Glomerella* isolé des téguments de la capsule en général et de ceux de l'apex en particulier indique qu'il existe là une population parasite prête à entrer dans la capsule au moindre défaut d'étanchéité et à y provoquer une pourriture.

Enfin, la fréquence des formes *Glomerella* dans les isolements réalisés à partir de feuilles cotylédonnaires et leur multiplication avec la culture du cotonnier montrent que celles-ci peuvent assurer leur survie dans le sol d'une année sur l'autre.

CONCLUSION

Cette étude montre la grande diversité des formes de *Glomerella* et de *Colletotrichum* isolées du cotonnier mais reste descriptive et soulève deux questions fondamentales :

1. Quelles sont les formes pathogènes et les formes seulement parasites ?

2. Existe-t-il une filiation entre les différents types isolés ? En particulier, la forme typique *C. gossypii* résulte-t-elle de l'évolution d'une des formes *Glomerella* décrites ?

Une étude du pouvoir pathogène est nécessaire pour compléter ce travail en essayant d'apporter une réponse à ces deux questions.

RÉSUMÉ

Des isolements réalisés dans les différents organes du cotonnier indiquent l'existence d'une grande diversité de forme de *Colletotrichum* : la forme *C. gossypii* South., une forme à petites spores cylindriques, une forme à spores falciformes, et de *Glomerella* : quatre grands groupes dont deux possédant en plus la forme imparfaite et très voisins macroscopiquement de *C. gossypii* mais à conidies plus courtes (groupes III et IV).

La répartition est très différente suivant les organes et peut se résumer ainsi :

Graines

(Infection interne) : *C. gossypii* South.

Plantules

— Nécrosées : *C. gossypii*.

— Saines : *Glomerella* sp. (groupe I et II surtout) 70 à 95 % ; *C. gossypii* 5 à 20 % ; *Colletotrichum* sp. 0 à 10 %.

Plants

— Parties lignifiées : Isolement rare d'une quelconque de ces formes.

— Parties vertes (feuilles, pétioles, tiges non aoutées) : *Glomerella* sp. (groupes I et II surtout).

Capsules

— Infection interne des téguments : *Glomerella* sp. (groupes I et II surtout) ; *C. gossypii* plus rarement ; *C.* à spores falciformes : très rarement.

— Capsules momifiées et capsules présentant une pourriture externe : *Glomerella* sp. (groupes III et IV surtout) ; *C. gossypii* ; *C.* à spores falciformes . plus rarement.

Cette étude soulève deux problèmes importants : celui du pouvoir pathogène des différents organismes isolés et la filiation éventuelle de *C. gossypii* avec certains types de *Glomerella*.

SUMMARY

Some isolations realized from various cotton organs indicate the existence of a great diversity of Colletotrichum *forms : the* C. gossypii *South. form, a form with cylindrical small spores, a form with falciform spores, and* Glomerella *: four large groups two of which possessing besides the imperfect form and macroscopically very closely related with* C. gossypii *but with shorter conidia (groups III and IV).*

The distribution is very different according to the organs and can be summarized as follows.

Seeds

(Internal infection) : C. gossypii *South.*

Seedlings

— Necrosed : C. gossypii.

— Healthy : Glomerella *sp. (especially groups I and II)* 70 *to* 95 % ; C. gossypii 5 *to* 20 % ; *Colletotrichum sp.* 0 *to* 10 %.

Plants

— Lignified parts : Rare isolation of anyone of these forms.

— *Green parts (leaves, petioles, non lignified stems) :* Glomerella *sp. (especially groups I and II).*

Bolls

— *Internal infection of teguments :* Glomerella *sp. (especially groups I and II)* ; C. gossypii *more rarely* ; C. *with falciform spores : very rarely.*

— *Mummified bolls and bolls displaying external rot :* Glomerella *sp. (especially, groups III and IV)* ; C. gossypii ; C. *with falciform spores : more rarely.*

This study sets two important problems : that of the pathogenic power of various isolated organisms and the eventual filiation of C. gossypii *with some* Glomerella *types.*

RESUMEN

Aislamientos realizados en los diferentes órganos del algodonero indican la existencia de una gran diversidad de forma de Colletotrichum *: la forma* C. gossypii *South., una forma con pequeñas esporas cilíndricas, una forma con esporas falciformes, y de* Glomerella *: cuatro grandes grupos de los cuales dos poseen además la forma imperfecta y muy próximos macroscópicamente de* C. gossypii, *pero con conidios más cortos (groupos III y IV).*

La repartición es muy diferente según los órganos y puede resumirse así :

Simientes

(Infección interna) : C. gossypii *South.*

Plántulas

— *Con necrosis :* C. gossypii *South.*

— *Sanas :* Glomerella *sp. (Grupo I y II sobretodo)* 70 *a* 95 % ; C. gossypii 5 *a* 20 % ; Colletotrichum *sp.* 0 *a* 10 %.

Plantas

— *Partes lignificadas : Aislamiento raro de una cualquiera de esas formas.*

— *Partes verdes (hojas, pecíolos, tallos no madurados) :* Glomerella *sp. (Grupo I y II sobretodo).*

Cápsulas

— *Infección interna de los tegumentos :* Glomerella *sp. (Grupo I y II sobretodo)* ; C. gossypii *más raramente ;* C. *a esporas falciformes : muy raramente.*

— *C. momificados y C. presentando una podredumbre externa :* Glomerella *sp. (Grupo III y IV sobretodo)* ; C. gossypii ; C. *a esporas falciformes : más raramente.*

Este estudio plantea dos problemas importantes : el del poder patógeno de los diferentes organismos aislados y la filiación eventual de C. gossypii *con ciertos tipos de* Glomerella.

SUR LES DIFFÉRENTES FORMES DE *Glomerella* Spaul. et Schr. ET DE *Colletotrichum* Cda ISOLÉES DU COTONNIER *(Gossypium hirsutum* L.*)*

II. - Etude du pouvoir pathogène, premières conclusions

par

J. C. FOLLIN

Phytopathologiste

Station Centrale de BAMBARI

Ce travail a été réalisé en 1968 sur la Station de BAMBARI.

INTRODUCTION

Une étude précédente (5) a montré que plusieurs formes de *Glomerella* et de *Colletotrichum* coexistaient sur le cotonnier sinon avec des répartitions, tout au moins, semble-t-il, avec des spécialisations parasitaires particulières. Cette étude amenait à poser le problème des formes parasites et des formes pathogènes ainsi que celui de la filiation entre les différents types. C'est dans cette optique que fut réalisée une série d'inoculations expérimentales sur plantules et sur capsules à partir de souches représentant les différentes formes isolées.

MATÉRIEL ET MÉTHODE

A) Infection des plantules

1 - *Infection sur banc de sable*

Les graines délintées à l'acide sulfurique puis triées sont immergées 6 h dans une suspension dans l'eau stérile de spores et de mycélium (broyage ménagé de 2 tubes à essai dans 200 cc d'eau); elles sont ensuites repiquées sur banc de sable stérile (fig. 1). L'observation des résultats se fait 6 jours après le semis. Cette méthode est utilisée pour avoir une réponse rapide sur l'existence ou la non-existence de pouvoir pathogène d'une souche et pour tester les réactions variétales de cotonniers à l'anthracnose. Dans ce dernier cas, l'appréciation des résultats est faite en calculant la mortalité réelle par la correction d'Abott :

$$Mr = \frac{100\ (Mo - Mt)}{100 - Mt}$$

Mr = mortalité réelle ;
Mo = mortalité observée ;
Mt = mortalité du témoin.

2 - *Infection en tubes*

Les graines délintées à l'acide sulfurique sont triées, désinfectées au bichlorure de mercure à 0,1 % pendant 10 minutes puis mises à germer 24 heures entre deux couches de coton hydrophile imbibé d'eau stérile. Les graines ayant normalement germé sont repiquées en tube sur milieu gélosé (a).

L'infection est faite lorsque les plantules ont 6 jours en ajoutant 1 ml d'une suspension de spores et de mycélium ; les résultats sont appréciés en calculant chaque jour les pourcentages cumulés de plants morts sur 30 plants inoculés (fig. 2).

B) Infection de capsules

1 - *Infection par pulvérisation*

Les capsules sont soit mises en bocaux (fig. 3) (8), soit fixées après désinfection pendant 5 mn au bichlorue de mercure sur un plateau en grillage maintenu au-dessus d'un bac rempli d'eau et entouré d'une gaine en polyéthylène permettant de garder une humidité relative voisine de 100 % (fig. 4). L'infection est faite par pulvérisation de spores sur les capsules.

2 - *Infection par piqûres*

Elle est réalisée sur capsules au champ suivant la méthode utilisée depuis plusieurs années sur la station de BAMBARI (1). L'appréciation des résultats est faite en examinant après 8 jours chaque loge et en les classant dans une des quatre catégories :

0 : loge saine ;
1 : loge peu attaquée ;
2 : loge moyennement attaquée ;
3 : loge totalement détruite.

(a) Il s'agit de la solution de crone gélosée (14) dont la composition est la suivante : $K_2H\ PO_4$: 1 g - KCl : 7 g - $CaSO_4$: 2,5 g - $MgSO_4\ 7H_2O$: 2,5 g - $Ca_3\ (PO_4)_2$: 0,5 g - $FePO_4$: 0,5 g - KNO_3 : 2 g ; les sels sont mélangés dans un mortier, la solution est preparée en mettant 1,5 g de ce mélange dans 1 l d'eau, la gélose est ajoutée (20 g/l) et le tout autoclavé.

Après avoir fait la somme des chiffres obtenus pour chaque loge dans les capsules étudiées, on divise par le nombre de loges pour avoir le degré de symptômes internes ; ce résultat est complété par le calcul du nombre de loges attaquées divisé par le nombre de capsules inoculées, il traduit la faculté pour un parasite de passer d'une loge à l'autre.

C) Souches utilisées

D'une manière générale, on préfère prendre des souches présentant une forme imparfaite, ceci permettant un inoculum plus homogène.

1 - *Inoculation des plantules*

— Sur sable : tous les types de *Glomerella* et *Colletotrichum* rencontrés.

— En tubes : *Glomerella*, 1 souche de chacun des groupes I, II, III et IV, la souche IV a été inoculée sous sa forme (+) et sa forme mutante (—) (4, 6).

Colletotrichum, souche typique et souche à spores falciformes.

2 - *Inoculation des capsules*

— Par pulvérisation : *Colletotrichum gossypii* et forme IV de *Glomerella* (isolée en 1967 de lésions typiques sur capsules).

— Par piqûres : 1 souche des groupes I, II, III et IV et 1 souche de *C. gossypii*.

Toutes les souches utilisées proviennent de clones d'origine monospore.

D) Terminologie

Le mot parasite est employé dans son sens strict, c'est-à-dire qu'il définit un organisme capable de vivre aux dépens d'un hôte vivant, le mot pathogène désigne un parasite pouvant déterminer une maladie, la virulence est une faculté commune aux parasites et aux pathogènes mais à des degrés différents. Le pouvoir pathogène est l'apanage des organismes pathogènes.

RÉSULTATS

Inoculation des plantules

1) Les inoculations par trempage des graines puis semis sur sable donnent des résultats nets : les souches imparfaites typiques à spores élancées produisent des infections très fortes. Une étude en cours sur les réactions variétales donne une idée des mortalités couramment obtenues (tableau 1). Les formes parfaites donnent toujours, par contre, des résultats négatifs même en utilisant des temps de trempage plus longs et en les complétant par arrosage de spores en suspension dans l'eau. Les formes imparfaites à spores courtes et à spores falciformes ont également donné des résultats négatifs.

Nous n'avons pas eu de problèmes avec la conservation du pouvoir pathogène des souches ; la souche de référence isolée de graines conserve une virulence après 1 an de repiquages successifs sur décoction de haricot de Lima gélosé (lima bean agar Difco). Pour obtenir des infections fortes, il suffit de prendre la précaution d'utiliser des cultures jeunes de 8-10 jours et d'assurer une humidité suffisante.

Tableau 1. — *Mortalité obtenue par inoculation de* C. gossypii *sur différentes variétés de* Gossypium hirsutum

Variétés	Mortalité réelle %
D 9	88,4
B 50	78,0
BJA 592	69,5
Allen 333	88,4
BTK 12	85,7
SW 296	75,4
HG 9	93,7

2) Les inoculations sur plantules en tube sur milieu gélosé ont permis de chiffrer ces différences dans la virulence des différentes souches. Après le repiquage, les cotonniers ont une croissance normale pendant 5 à 6 jours puis restent ensuite bloqués au stade deux feuilles ; la limite de cet arrêt de croissance semble ne dépendre que du milieu qui peu à peu se dessèche. Une étude réalisée dans le but de mettre cette technique au point a montré que l'on pouvait conserver ainsi ces plantules sans dommage pendant 2 mois.

— Dans ces conditions tout à fait artificielles, il est bien évident que la plante ne peut compenser par sa croissance les dégâts causés par un organisme parasite et que des souches non pathogènes dans les conditions naturelles peuvent provoquer des symptômes morbides. Le temps nécessaire pour entraîner cette mortalité nous sert alors de critère pour différencier les souches.

Tableau 2. — *Temps minimum pour obtenir* 50 % *de mortalité* (*TL* 50) *et intervalle entre le début de l'attaque et les* 100 % *de mortalité*

Souches	T.L. 50 (jours)	Int. (0-100) (jours)
C. gossypii	7	4
Glomerella		
I	18	8
II	17	8
III	18	8
IV +	18	7
IV —	19	9
C. à spores falciformes	20	11

PLANCHE I

Fig. 1. — Plantules issues de graines infectées par *C. gossypii* (à droite) et plantules témoins.

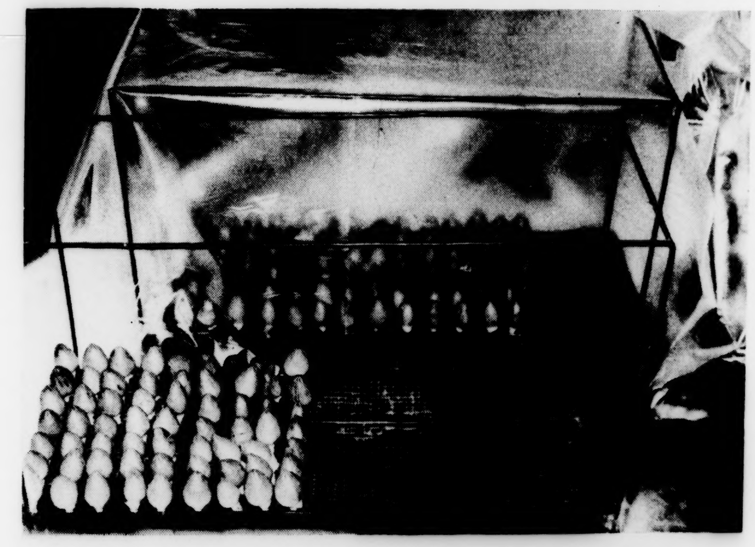


Fig. 4. — Capsules en bacs recouverts de polyéthylène infectées par pulvérisation d'une solution de spores.

Fig. 5. — Infection d'une capsule par piqûre.

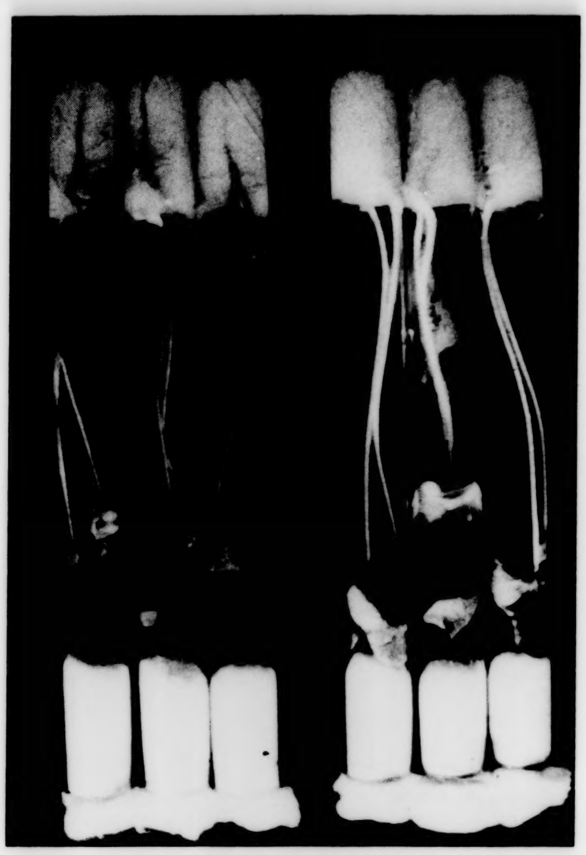
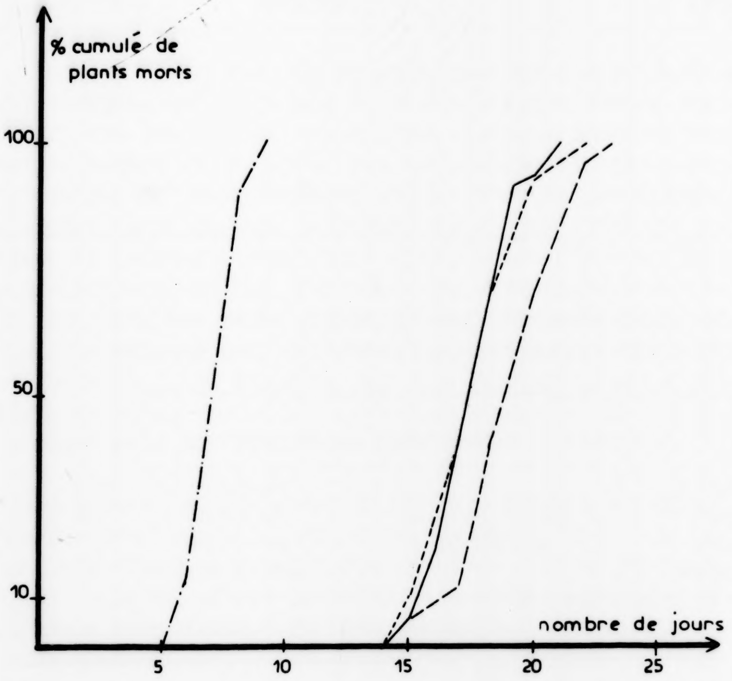


Fig. 2. — Infection de plantules en tubes - à droite infection par *C. gossypii*, à gauche, plantules témoins.

Fig. 3. — Capsule infectée au laboratoire par pulvérisation d'une solution de spores.

A gauche, le micropulvérisateur Vaast.

— Le tableau 2 et le graphique n° 1 confirment les résultats des inoculations sur banc de sable. Seule la forme typique *C. gossypii* cause une mortalité précoce et rapide ; aucune plantule du témoin ne s'est flétrie pendant toute la durée de cette expérience. Dans ces conditions artificielles, la virulence de la forme imparfaite typique est nettement plus forte.



Graphique 1. — Courbes représentatives des pourcentages cumulés de mortalité causée par *C. gossypii* (— - —) et deux souches de *Glomerella* dont une sous la forme (+) (——) et sous la forme (—) (- - - -).

Inoculation des capsules

1) *Par pulvérisation.* La méthode isolant les capsules en bocal est la meilleure et a donné 100 % d'infection avec la souche typique et 0 % avec la forme *Glomerella*, mais le faible nombre de capsules pouvant être inoculé limite la valeur des résultats ; la méthode en bacs recouverts de polyéthylène confirme ces derniers. Il est à noter que la forme *Glomerella* n'attaque pas les capsules même blessées superficiellement (tableau 3), sa présence au champ sur lésions de capsules résulte vraisemblablement d'une blessure profonde permettant à ces organismes présents dans les téguments de pénétrer à l'intérieur de la capsule et de provoquer une pourriture interne puis une pourriture externe qui se traduit par une lésion typique d'anthracnose couverte d'acervules.

2) *Par piqûres.* Les résultats (tableau 4) montrent qu'après 9 jours d'incubation les quatre souches de *Glomerella* ont à peine endommagé plus d'une loge alors que *C. gossypii* a détruit entre 2 et 3 loges. Par piqûre également, les dégâts provoqués par la forme imparfaite typique sont plus importants.

DISCUSSION

Dans une étude précédente (5), nous posions en conclusion 2 questions :

— Quelles sont les formes pathogènes et les formes seulement parasites ?

— Existe-t-il une filiation entre les différents types de *Glomerella* et de *Colletotrichum* isolés ?

En ce qui concerne la première question, la réponse est nette : *Colletotrichum gossypii* South. est seul pathogène, les formes *Glomerella* et la forme *Colletotrichum* à spores falciformes doivent être considérées comme des parasites secondaires ce qui ne diminue d'ailleurs pas leur importance car elles sont fréquemment trouvées sur lésions typiques externes de capsules. Cette infection est très certainement consécutive à une blessure accidentelle, nous pensons surtout aux déprédations d'insectes en particulier aux dégâts des *Dysdercus* ; en définitive, pour le cas particulier des capsules, le milieu extérieur est plus important que les capacités du champignon parasite lui-même.

Les différences dans les aptitudes parasitaires des différentes formes suggèrent par ailleurs une explication aux déboires relevés dans certaines études (2) lorsque des souches infectant le cotonnier à l'état latent sont utilisées pour des inoculations artificielles de plantules. Ce sont en effet des formes *Glomerella* dans la très grande majorité des cas que l'on isole des parties internes et, sans une certaine habitude, la confusion est facile entre certaines formes parfaites (groupes III et IV en particulier) et la forme imparfaite typique.

Cette situation présente une analogie certaine avec celle de l'anthracnose du caféier. A ce sujet, deux séries d'études sont particulièrement intéressantes : celles de NUTSMAN et ROBERT (12) et celle plus récente de HOCKING (9). Leurs conclusions sont identiques sur les distinctions entre les différentes souches isolées : une forme *Colletotrichum, Colletotrichum coffeanum* Nov. dont le stade parfait n'a jamais été observé provoque l'anthracnose typique (coffee berry disease), entre autres cette forme est seule capable d'attaquer les baies vertes ; une autre forme également nommée *Colletotrichum coffeanum*, différente d'après HOCKING en culture, possède une forme parfaite *Glomerella* fréquemment obtenue, elle possède une virulence très faible, ses attaques se limitent aux baies mûres et aux tissus moribonds (Brown blight disease), elle peut infecter les feuilles à l'état latent sans provoquer de nécroses.

Par ailleurs, RAYNER (13) mentionne l'existence en infection latente de deux types de *Colletotrichum* : un type à spores courtes trouvé généralement sur les rameaux et les feuilles, non pathogène, et un type à spores plus longues fréquemment isolé de baies vertes, pathogène ; c'est à ce dernier type que RAYNER rattache l'agent du coffee disease.

Une situation semblable se rencontre dans le cas

Tableau 3. — *Pourriture externe obtenue par pulvérisation de spores de* C. gossypii *et* Glomerella *sur capsules de cotonnier*

	Capsules en bocaux		Capsules en bacs			
	Capsules entières		Capsules entières		Capsules blessées superficiellement	
	Nombre de capsules	% de C. pourries	Nombre de capsules	% de C. pourries	Nombre de capsules	% de C. pourries
Glomerella	6	0	63	0	70	0
C. gossypii	6	100	56	21,4	56	100
C. à spores falciformes	—	—	50	0	50	0
Témoin	—	—	49	0	38	0

Tableau 4. — *Degré moyen d'attaque et nombre de loges pourries par capsules résultant de l'inoculation de* Glomerella *sp. et* C. gossypii - 2 x 100 *capsules sont inoculées par souche*

Souches	Incubation de 9 jours		Incubation de 15 jours	
	Degré moyen d'attaque	Nombre de loges pourries par capsule	Degré moyen d'attaque	Nombre de loges pourries par capsule
Glomerella				
I	0,7	1,4	0,8	1,5
II	0,6	1,1	0,7	1,3
III	0,7	1,3	0,8	1,4
IV	0,6	1,2	0,8	1,5
C. gossypii	1,1	2,4	1,8	3,3

d'une pourriture à *Fusarium* des cabosses de cacaoyer. L'étude réalisée par FORD, BOURRET et SNYDER (7) montre que :

1° - les souches présentant en culture la forme imparfaite (*Fusarium rigidiusculum*) (Brick) Snyd. et Hans) et la forme parfaite (*Calonectria rigidiuscula* (Berk. et Brme) Sacc.) ne sont pas pathogènes.

2° - Les souches pathogènes sont toutes des formes imparfaites.

3° - Certaines souches imparfaites pathogènes donnent lorsqu'on les confronte une ligne de périthèces.

Les deux premiers points sont conformes aux résultats de cette étude ; le troisième point n'a pas été étudié mais il est probable que, compte tenu de la sexualité particulière des *Glomerella*, la situation soit différente.

Ceci amène à la deuxième question posée par cette étude : le problème de la filiation *Glomerella* - *Colletotrichum gossypii* ; en effet, la première hypothèse pour tenter d'expliquer cette situation est que certaines formes *Glomerella* infectent à l'état latent le cotonnier et que, brusquement, pour une raison non déterminée une transformation fait apparaître à partir de ces formes un type pathogène déclenchant la maladie, la disparition de la forme sexuée pouvant être considérée comme une simplification de l'organisme liée à un parasitisme plus fort. Il est certain que dans le cas du *Fusarium* du cacaoyer une adaptation au parasitisme a fait disparaître la forme sexuée, la démonstration en est faite par le recouvrement de cette forme par croisement permettant par complémentation de reconstruire la chaîne des réactions métaboliques nécessaires à la formation du périthèce. En ce qui concerne *Glomerella*, une étude précédente (6) sur le déterminisme de la forme sexuée montre que les formes sauvages de *Glomerella* sont, *in vitro*, très instables pouvant évoluer vers une forme *Colletotrichum* sur le simple blocage d'un gène ; il est donc parfaitement possible que la forme imparfaite soit le résultat d'une évolution d'une forme parfaite vers la pathogénicité. Il n'a cependant pas été possible de rattacher ni morphologiquement ni sexuellement *Colletotrichum gossypii* à un type de *Glomerella* ; à ce dernier titre, toutes les souches de *Glomerella* ont d'ailleurs montré une individualité parfaite et il n'a pas été possible de réaliser des croisements entre formes + et — de souches strictement identiques morphologiquement et isolées par-

fois du même organe alors que ces croisements sont aisés entre mutants d'une même forme sauvage. D'autre part, si toutes les formes parfaites isolées sont dénuées de tout pouvoir pathogène, la réciproque n'est pas vrai et certaines souches imparfaites à petites spores ne sont pas non plus pathogènes, (ce type se rattache d'ailleurs morphologiquement aux formes *Glomerella* du groupe IV) ; enfin, les souches conidiennes mutantes de deux souches du groupe IV ne sont pas pour autant devenues pathogènes.

Tout ceci indique, d'une part, que s'il est probable qu'une adaptation puisse faire évoluer une forme parfaite vers une forme imparfaite, il s'agit plutôt d'une conséquence et il n'y a pas de liaison étroite entre le mode de reproduction et le pouvoir pathogène, d'autre part qu'une filiation n'est pas évidente et rien ne permet d'affirmer que *Colletotrichum gossypii* apparaisse par mutation d'une forme *Glomerella* décrite dans cette étude. *Glomerella cingulata* est un champignon courant dans les pays à climat chaud et humide, il est possible que ces formes soient d'origine locale et que *Colletotrichum gossypii* ait été introduit avec les premières graines de cotonnier.

Le problème des formes *Colletotrichum* à petites spores semblables morphologiquement à la forme imparfaite des *Glomerella* des groupes III et IV reste posé : s'agit-il d'une première évolution vers une virulence plus forte ? Et ces parasites peuvent-ils un jour fournir des pathogènes par mutation ou par échange parasexuel, entre eux ou avec la forme pathogène typique ?

CONCLUSION

Cette étude réalisée à l'origine dans le but d'étudire l'anthracnose du cotonnier à partir des formes parfaites de *C. gossypii* a finalement montré l'existence de différents types très voisins et l'absence de pouvoir pathogène des formes parfaites. Ces différences peuvent permettre de douter de la réalité de l'existence de l'espèce *Glomerella gossypii* Edg. qui a d'ailleurs été souvent critiquée. LEAKY et PERRY (11) après une étude taxonomique très complète concluent à la synonymie entre *G. cingulata* et *G. gossypii* d'après la description d'EDGERTON (3) et les premiers émettent des doutes sur l'existence d'une forme parfaite de *C. gossypii.* Par ailleurs, l'étude présente ne démontre pas une liaison physiologique entre aucune des formes *Glomerella* décrites et le cotonnier. On ne peut encore parler de formes spécialisées et celles-ci doivent être considérées comme différents types de *Glomerella cingulata* (Ston.) Sp. et Von Schr. dans le sens de VON ARX et MÜLLER (16).

Si les caractères taxonomiques ne permettent pas de maintenir *G. gossypii* comme une espèce différente de *G. cingulata*, l'étude d'EDGERTON ne permet pas non plus de conclure à l'existence d'une forme parfaite de *C. gossypii ;* celle-ci, en effet, ne mentionne pas de test de virulence avec des souches monospores et décrit ce stade présumé parfait de l'anthracnose sur *Gossypium herbaceum* et non sur *G. hirsutum* comme on pourrait s'y attendre. La même objection quant à l'absence de test de virulence peut être faite pour la dernière note signalant le stade parfait de *Colletotrichum gossypii* (10) ; la présence de périthèce de *Glomerella* sur une capsule mise à incuber suffit à annoncer l'existence de *Glomerella gossypii.* L'étude présente montre que si effectivement la présence de *Glomerella* est fréquente sur capsules, même sur capsules portant des lésions caractéristiques d'anthracnose, il est hâtif de conclure qu'il s'agit de la forme parfaite de *Colletotrichum gossypii* et les tests de virulence réalisés à partir de souches monospores montrent au contraire qu'il s'agit vraisemblablement de champignons différents. Si une description morphologique est nécessaire, elle est rarement suffisante et seule une étude du pouvoir pathogène peut décider de l'identité de deux organismes ; c'est un principe de base souvent peu respecté.

En définitive, le problème souvent posé de la synonymie entre *G. gossypii* Edg. et *G. cingulata* Ston. (Sp. et Schr.) n'est probablement pas à envisager car l'existence d'une forme *Glomerella* pathogène du cotonnier n'a jamais été réellement démontrée. Toutes les études concernant l'anthracnose du cotonnier font, par contre, état de l'utilisation de formes imparfaites, en particulier l'étude réalisée par ULLSTRUP (15) sur les « variations chez *Glomerella gossypii* » ne mentionne aucune forme parfaite ; c'est pourquoi nous pensons que, jusqu'à preuve du contraire, la dénomination *Colletotrichum gossypii* South. est la seule valable. Les similitudes existant entre cette forme et certaines formes de *Glomerella* parasites du cotonnier doivent cependant retenir l'attention et l'existence d'une forme sexuée peut permettre d'analyser certains phénomènes communs.

RÉSUMÉ

Quatre formes de *Glomerella* et deux formes de *Colletotrichum* (*C. gossypii* South. et *C.* à spores falciformes) isolées du cotonnier de nécroses de racines (*C. gossypii*), de nécroses de capsules (*C. gossypii, C.* à spores falciformes et *Glomerella* sp.) et d'infection latente (*C. gossypii* et *Glomerella* sp.) sont étudiées sur plantules et sur capsules pour leur pouvoir pathogène.

Seule la forme typique *Colletotrichum gossypii* South. est pathogène sur plantules et sur capsules quelque soit le mode d'infection.

Les formes *Glomerella* et la forme *Colletotrichum* à spores falciformes ne provoquent des pourritures sur capsules que si ces organismes sont introduits par piqûres.

Ces résultats sont analysées compte tenu d'études précédentes réalisées en particulier sur l'anthracnose du caféier *arabica.* Une filiation entre la forme typique *C. gossypii* et certains types de *Glomerella* très voisins morphologiquement n'est pas évidente ; la dénomination *Glomerella gossypii* Edg. peut faire l'objet de critique.

BIBLIOGRAPHIE

1. CAUQUIL J. et P. MILDNER, 1965. — Première étude sur le comportement variétal du cotonnier en présence des pourritures de capsules. *Cot. Fib. trop.*, 20, 4, 539-548.

2. COGNEE M. — Rapport de la Section de Phytopathologie de la Station de l'I.R.C.T. à Bouaké, 1960-61 et 1961-62.

3. EDGERTON C.W., 1909. — The perfect stage of the cotton anthracnose. *Mycologia*, 1, 114-120.

4. EDGERTON C.W., 1913. — Plus and minus strains in the genus *Glomerella*. *Amer. J. Bot.*, 1, 244-254.

5. FOLLIN J.C., 1969. — Sur les différentes formes de *Glomerella* et de *Colletotrichum* isolées du cotonnier. I. Localisation, étude morphologique. *Cot. Fib. trop.*, 24, 3.

6. FOLLIN J.C., 1968. — Sur le déterminisme de la formation du périthèce chez *Glomerella cingulata* f. sp. *gossypii*. *Cot. Fib. trop.*, 23, 4, 447-451.

7. FORD E.J., J.A. BOURRET et W.C. SNYDER, 1967. — Biologic specialization in *Calonectria* (*Fusarium*) *rigidiuscula* in relation to green point gall of Cocoa. *Phytopath.*, 57, 710-712.

8. HALISKY P.M., W.C. SCHNATHORST et M.A. SHAGRUN, 1961. — Severity and distribution of cotton bolls rots as related to temperature. *Phytopath.*, 51, 8, 501-505.

9. HOCKING D., 1966. — Brown blight (*Colletotrichum coffeanum* Noak) of arabica coffea in East Africa. *Ann. appl. Biol.*, 58, 409-421.

10. KURIAN N.J. et L. MOMIZ, 1966. — Perfect stage of *Colletotrichum gossypii* in Indian. *Indian Phytopath.*, 19, 4, 383-384.

11. LEAKY C.L.A. et D.A. PERRY, 1966. — The relation between damage caused by insect pests and boll rot associated with *Glomerella cingulata* (Ston.) Sp. et Schr. (*Colletotrichum gossypii* South.) on Upland Cotton in Uganda. *Ann. appl. Biol.*, 57, 337-344.

12. NUTSMAN F.J. et F.M. ROBERTS. — Investigations on a disease of *Coffea arabica* caused by a form of *Colletotrichum coffeanum* Noack.
I. Some factors affecting infection by the pathogen. *Trans. Brit. myc. Soc.*, 43, 3, 489-505. 1960.
II. The relation between infection of bearing wood and disease incidence. *Trans. Brit. myc. Soc.*, 44, 3, 511-521, 1961.

13. RAYNER R.W., 1948. — Latent infection in *Coffea arabica*. *Nature, Lond.*, 161, 245-246.

14. ROVIRA A.D., 1956. — Plant root excretions in relation to the rhizosphere effect. *Plt and Soil*, 7, 178-217.

15. ULLSTRUP A.J., 1938. — Variability of *Glomerella gossypii*. *Phytopath.*, 28, 787-798.

16. VON ARX J.A. et E. MULLER, 1954. — Die Gattungen der amerosporen Pyrenomyceten. *Breitr. Kryptoflora Schweiz*, 11, 1.

SUMMARY

Four Glomerella *forms and two* Colletotrichum *forms* (C. gossypii *South. and* C. *with falciform spores*) *isolated from cotton, from root necroses* (C. gossypii), *boll necroses* (C. gossypii, C. *with falciform spores and* Glomerella *sp.*) *and from latent infection* (C. gossypii *and* Glomerella *sp.*) *are studied on seedlings and on bolls in relevance with their pathogenic power.*

Only the Colletotrichum gossypii *South. typical form is pathogenic on seedlings and on bolls whatever the mode of infection.*

The Glomerella *forms and the* Colletotrichum *form with falciform spores induce boll rot only if these organisms are introduced by punctures.*

These results are analysed taking in consideration earlier studies pursued in particular on anthracnose of Arabica *coffee ; a filiation between the* C. gossypii *typical form and some* Glomerella *types very closely related morphologically is not evident. The denomination of* Glomerella gossypii *Edg. may form the object of criticism.*

RESUMEN

Cuatro formas de Glomerella *y dos formas de* Colletotrichum (C. gossypii *South. y* C. *a esporas falciformes*) *aislados del algodonero de necrosis de raíces* (C. gossypii), *de necrosis de cápsulas* (C. gossypii, C. *a esporas falciformes y* Glomerella *sp.*) *y de infección latente* (C. gossypii *y* Glomerella *sp.*) *se estudian sobre plántulas y sobre cápsulas por su poder patógeno.*

Solo la forma típica Colletotrichum gossypii *South. es patógena sobre plántulas y sobre cápsulas cualquiera que sea el modo de infección.*

Las formas Glomerella *y la forma* Colletotrichum *a esporas falciformes no provocan podredumbres en las cápsulas a no ser que esos organismos sean introducidos por inyecciones.*

Estos resultados se analizan teniendo en cuenta estudios precedentes realizados en particular sobre la antracnosis del cafeto arabica ; una filiación entre la forma típica C. gossypii *y ciertos tipos de* Glomerella *muy próximos morfológicamente no es evidente, la denominación* Glomerella gossypii *puede ser objeto de crítica.*

ORIGINE ET
MISE EN PLACE DES FIBRES DANS LES PARTIES AÉRIENNES DE L'*Hibiscus cannabinus* (Malvacées) [1]

par

J. BOURÉLY

NOTE DE L'AUTEUR.

— *Une connaissance plus précise de l'origine et de la mise en place des fibres textiles dans les parties aériennes de l'*Hibiscus cannabinus *nous a paru nécessaire avant d'entreprendre, dans le cadre même des Etudes menées à l'I.R.C.T., des travaux de portée plus directement pratique et susceptibles, en particulier, d'être exploités dans les différents secteurs d'amélioration de la production des fibres (agronomie, génétique, technologie).*

— *Cette étude a été réalisée au Laboratoire de Madame* Nougarède - *Professeur de Botanique - Laboratoire de Cytologie et Morphogenèse Végétales, 9, quai Saint-Bernard - 75 - Paris-5*e

L'*Hibiscus cannabinus* L. (Malvacées) est une plante textile tropicale dont la tige au port dressé (2,5 à 3 m de hauteur) fournit une fibre utilisable en sacherie en remplacement de celle du Jute. Nous nous proposons, dans cette étude, de déterminer l'origine et la mise en place des différents types de fibres, dans l'hypocotyle et l'épicotyle de la variété « I.R.C.T.. Soudan Précoce ». Pour réaliser ce travail, nous suivrons les modalités de différenciation des tissus depuis la graine jusqu'à l'édification de la dix-septième feuille produite par le point végétatif (50 cm de haut). Nous utiliserons les techniques anatomiques classiques et essentiellement des sections transversales pratiquées à divers niveaux.

Développement des fibres dans l'hypocotyle

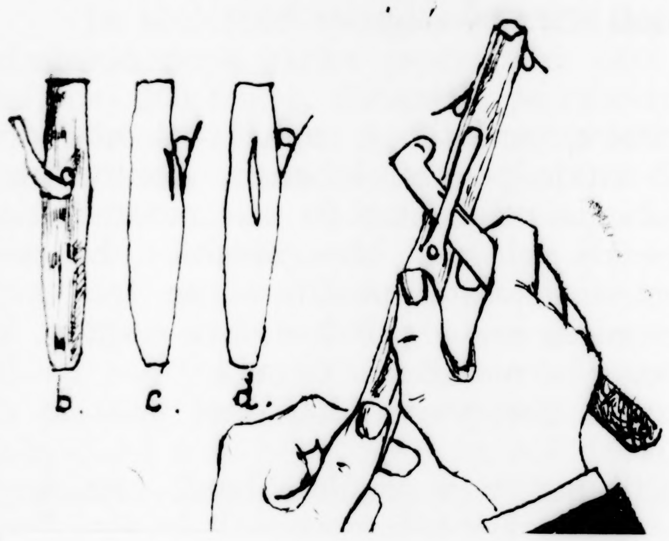
Les figures 1 et 2 donnent l'aspect morphologique de germinations depuis 24 h jusqu'à 144 h. Dès 24 h de germination, alors que la plantule est encore incluse dans la graine (fig. 1 a), les premiers éléments de xylème (fig. 3, xy) et de phloème (fig. 3, ph) se différencient dans l'hypocotyle à 200 µ du point végétatif. Après 48 h, (fig. 4 a et b), on distingue, à la base de l'hypocotyle, au niveau marqué, ab, sur la figure 1b, quatre ou parfois cinq massifs de xylème primaire, xy, qui alternent avec ceux du phloème primaire, ph. Après 200 h de germination, ces derniers (fig. 5, ph) sont eux-mêmes segmentés par des cellules de parenchyme, pl, en cinq ou six « sous-massifs » phloémiens, de six à douze cellules chacun, en section transversale. A l'intérieur d'un même massif de phloème ainsi subdivisé en sous-massifs (soulignés par les flèches épaisses), ce sont les sous-massifs les plus internes dont les cellule épaississent et lignifient leurs parois (fig. 5, flèches fines); les cellules des sous-massifs les plus externes, situées près du xylème, demeurent plus longtemps non épaissies.

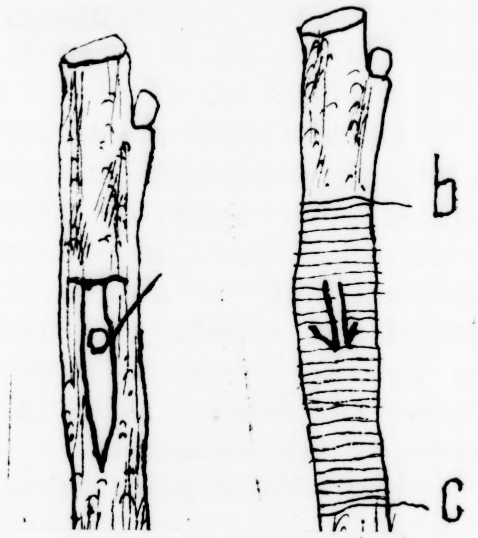
Ainsi, les phénomènes d'épaississement progressent depuis les sous-massifs situés au centre du massif phloémien jusqu'aux sous-massifs proches du xylème. Dès ce stade (fig. 5), un cambium, c, s'est déjà formé. Après 220 h de germination (fig. 6 a), un très grand nombre de cellules de phloème se sont déjà transformées en fibres lignifiées, fp, dans chaque sous-massif. *Ces fibres d'origine primaire et phloémienne sont les premières formées.* Elles sont responsables de l'extrême dureté de l'hypocotyle. Ultérieurement (fig. 6 b), toutes les cellules des massifs de phloème à l'exception du parenchyme, pl, épaissiront leurs parois. Le cambium mis en place au tout début de la germination (150 h) produit en direction de

---

(1) Note présentée à l'Académie des Sciences de Paris et parue dans les *C.R. Acad. Sc. Paris*, Série D, t. 268, p. 1714-1717 (31 mars 1969).

les ca ochiului să i se lase ţesutul generator de la bază fără de care prinderea este compromisă (această greşală se face mai ales la altoirea în ochi crescînd, fiindcă la cea în ochi dormind se lasă puţin lemn şi deci ţesutul generator este asigurat).





1969

RAFAILĂ, C. & COSTACHE, M.

Combaterea ofilirii Castravetilor în seră.
[Control of Cucumber wilt in the glasshouse.]

Revtă Hortic. Vitic., 18 (5):73-76. [4 fig.]

Microfilm no. 15621

PROTECȚIA CULTURILOR HORTI-VITICOLE

Combaterea ofilirii castraveților în seră

Dr. C. RAFAILĂ, biolog M. COSTACHE
Institutul de cercetări pentru protecția plantelor

Economicitatea culturilor de castraveți atît în cîmp cît mai ales în seră este frecvent afectată din cauza pierderii unui mare procent de plante pe toată durata perioadei de vegetație, ca urmare a căderii răsadului, putrezirii bazei tulpinii și ofilirii plantelor.

Simptomele caracteristice fenomenelor de ofilire sînt următoarele:

1) Imediat după repicare în ghivecele nutritive începînd din a 2-a sau a 3-a zi plăntuțele se înmoaie de la punctul de contact cu solul în sus, se apleacă, se veștejesc (fig. 1,2). Cauzele acestei ofilirii timpurii sînt datorate infecțiilor cu ciuperci patogene din genurile Pythium și Fusarium. Fenomneul se întîlnește mai rar în unitățile agricole care în loc de ghi-

Fig. 1

vece nutritive folosesc ghivece din material plastic (serele I.A.S. Arad, Oradea ş.a.).

2) Plantele de castraveţi în seră, după transplantare, manifestă înainte de ofilire o degradare progresivă a bazei tulpinii, fenomenul de ofilire manifestîndu-se brusc (de la o zi la alta) şi aceasta coincide cu putrezirea tulpinii la bază unde rămîn nealterate numai fascicolele vaselor de lemn (fig. 3). Din plantele ofilite s-au făcut de asemenea izolări repetate şi în toate cazurile s-au găsit ciuperci din genul Fusarium.

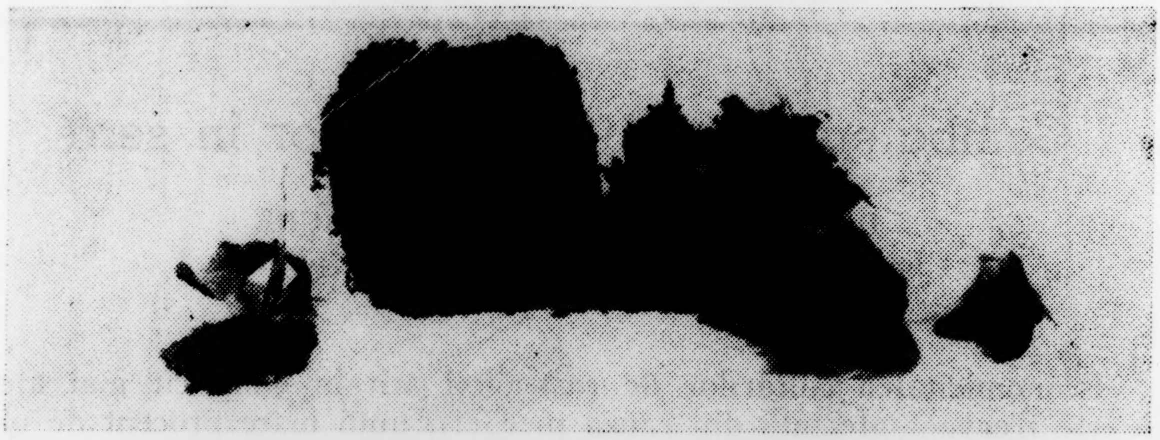


Fig. 2

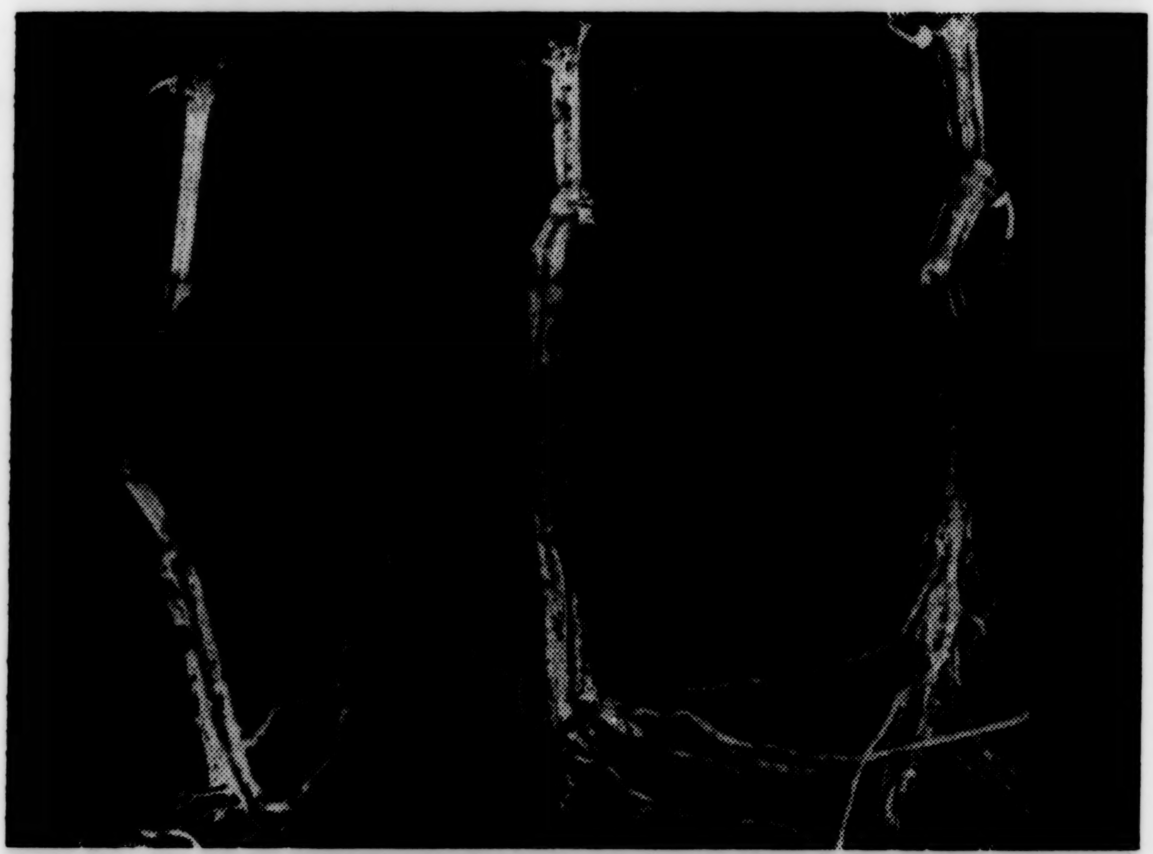


Fig. 3

În urma experienţelor executate la serele I.A.S. „30 Decembrie" şi a recomandărilor făcute la numeroase alte sere, s-au obţinut rezultate pozitive, ofilirea putînd fi înlăturată total sau se poate realiza o micşorare simţitoare a pagubelor, dacă simtomele sînt într-o fază incipientă.

Pentru aceasta se recomandă următoarele măsuri :

— semințele de castraveți se vor analiza dacă prezintă spori de ciuperci parazite sau saprofite și în caz de infecții, se vor supune unui nou tratament pentru care se recomandă cufundarea într-o soluție de sublimat 1‰ timp de 2—3 minute după care se spală bine cu apă distilată sau direct de la robinet. Nu se vor spăla semințele cu apă care a stat timp îndelungat în recipente, vase, în seră etc. Semințele după spălare se usucă într-un loc ferit de posibilitatea unor infecții (în camere izolate), acoperite cu hîrtie de filtru sau sugativă nouă ;

— pentru producerea răsadurilor de castraveți este necesară o suprafață de seră amenajată special ca înmulțitor, cu solul, scheletul și suprafața sticlei în interior bine dezinfectată. Cea mai bună dezinfecție a solului se poate realiza cu aburi sau apă fierbinte astfel ca solul să ajungă la o temperatură de 85—95°C, timp de cel puțin 30—45 minute. În cazul că nu se poate dezinfecta termic, se poate folosi o dezinfecție chimică cu captan 500 kg la ha.

Scheletul și sticla se dezinfectează printr-o stropire ușoară cu soluție de formalină 2% trecută prin sistemul de ploaie artificială.

Practica producerii răsadurilor în corpurile mari de seră, fără spațiu înmulțitor izolat, este defectuoasă și prezintă riscuri de infectarea plantelor din primul moment ;

— patul germinativ trebue să se facă din materiale dezinfectate termic. Nu se va utiliza turbă, nisip și pămînt care au mai fost folosite sau care au stat timp îndelungat în jurul serelor mai ales cînd amestecul nu poate fi dezinfectat termic. Peliculele de polietilenă, folosite adesea sub patul germinativ și pe strat după semănat, trebue să fie noi, neutilizate, sau dacă s-au mai folosit să fie supuse dezinfecției cu formalină. Uneltele cu care se amenajează straturile de la paturile germinative se dezinfectează de asemenea.

Lucrătorii care seamănă sămînța și îngrijesc de răsaduri trebuie să aibă mîinile spălate și dezinfectate cu o soluție slabă de permanganat de potasiu, iar îmbrăcămintea curată și chiar dezinfectată.

Folosirea muncitorilor din înmulțitoare la alte lucrări cu plante în sere și revenirea lor în înmulțitor este dăunătoare.

Patul germinativ pînă la răsărire și după ce răsar plantele trebuie stropit la fiecare 3—4 zile cu o suspensie de Captan sau Faltan 0,2%. Ghivecele nutritive sau amestecul de pămînt folosit în ghivecele de plastic sau de lut ars vor fi supuse dezinfecției termice.

Mașina de confecționat ghivece nutritive cît și ghivecele de pămînt sau din material plastic refolosite trebuie dezinfectate cu o soluție de formalină 2%.

Repicatul se face cu cea mai mare atenție, astfel ca la dislocarea plăntuțelor din patul germinativ, să nu fie lezate rădăcinile. Imediat după repicare să se facă o stropire a plantelor și ghivecelor cu o suspensie de Captan 0,2—0,3%, operație care se repetă la fiecare 3—4 zile.

Plantele care cad trebuie strînse de mai multe ori pe zi și distruse. Lucrătorul care face această operație se va spăla pe mîini și se va dezinfecta înainte de a face alte lucrări în înmulțitor ;

— plantarea răsadului în seră se face de asemenea cu grijă evitînd pe cît este posibil ruperea rădăcinilor sau lezarea tulpinilor.

După plantarea în seră, săptămînal se aplică plantelor un tratament cu Captan sau Faltan 0,2—0,3%. De asemenea, se fac tratamente de îmbiere a bazei tulpinii. Se utilizează în acest scop Captanul — 0,2% la plantele tinere şi progresiv pînă la 0,5% cînd plantele ajung la înălţimea normală — la intervale de 10—12 zile sau zeamă bordeleză 0,5—2% la intervale de 14—15 zile.

Fig. 4

Pentru o îmbăiere bună a bazei tulpinii se scoate de la duză spiralul de dispersie a suspensiilor, obţinîndu-se astfel un jet mai compact.

În cazul cînd apare fenomenul de degradare a bazei tulpinii se aplică prăfuiri repetate cu un amestec compus din o parte Captan şi 3 părţi talc. Experienţele au dovedit că prin aceste prăfuiri fenomenul de putrezire este oprit iar ţesuturile degradate se regenerează (se calusează — fig 4).

Se va evita în toate cazurile formarea de depresiuni în jurul tulpinilor plantelor care favorizează băltirea apei. De asemenea, administrarea de îngrăşăminte organice nu se va face în contact direct cu tulpina.

Pe toată perioada de vegetaţie vor fi combătute insectele (miriapozi, musculiţa albă, afidele ş.a.) care pot vehicula infecţiile de la o plantă la alta.

La lichidarea culturii de castraveţi, plantele se vor smulge iar rădăcinile rămase în sol se strîng la mobilizarea solului. Înainte de un nou ciclu de cultură, solul se dezinfectează în toate serele termic sau chimic.

În cazuri izolate, pentru dezinfecţia solului s-au utilizat preparate pe bază de mercur. Este cunoscut că mercurul din aceste preparate migrează în plante şi în special în fructe iar această recoltă este improprie consumului.

Preparatele recomandate mai sus sînt cele care se găsesc curent în dotaţia complexelor de sere.

Pericolul cariilor de lemn şi de scoarţă în livezi

Dr. T. PERJU
Institutul agronomic Cluj

După cîte se ştie din literatură (1, 2, 6, 10), speciile comune de *Scolitidae* dăunătoare livezilor sînt ; cariul lemnului *(Anisandrus, Xyleborus* sau *Dryocoetes dispar F.)* şi carii — mic şi mare — de scoarţă *(Eccoptogaster* sau *Scolytus rugulosus* Ratz şi *E.* sau *S. mali* Bechst.), *(Scolytidae, Coleoptera).*

În literatura noastră de specialitate se găsesc puţine lucrări, care să trateze aspecte privind cunoaşterea modului de comportare şi măsurile de combatere a speciilor dăunătoare de carii din livezi. Lucrările de sistematică a insectelor (8), de semnalare a dăunătorilor plantelor agricole (7), de organizare şi îndrumare a acţiunilor de combatere a dăunătorilor privind apărarea livezilor nu amintesc nimic în legătură cu prezenţa sau măsurile de combatere a speciilor de carii.

După cum arată Balachowski (2), cariul lemnului *(Anisandrus dispar)* este răspîndit în toată regiunea paleareticā, de la Atlantic la Pacific, în Siberia, Regiunea Mediteraneană, nordul Africii, Orientul Apropiat şi Mijlociu.

După cercetările de biologie a acestei insecte efectuate de unii autori, cariul lemnului este dăunător mai ales livezilor tinere, întîlnindu-se pe pomii în plină dezvoltare. Această observaţie s-a confirmat şi în condiţiile din ţara noastră.

În literatura noastră de specialitate dăunătorul este descris în cîteva lucrări de Entomologie horticolă nefiindu-i consacrată însă nici o lucrare care să trateze diferitele aspecte legate de bioecologia şi măsurile de combatere a lui. Deşi acest dăunător are un regim polifag, el preferă speciile de pomi ca : mărul, părul, piersicul, caisul, întîlnindu-se frecvent şi pe unii arbori forestieri ca mesteacănul, alunul, plopul, mai puţin frecvent găsindu-se pe fag, stejar, frasin, nuc. După cum arată Spîrchez (10) în pădurile noastre produce mari pagube castanului comestibil.

În legătură cu biologia cariului de lemn, majoritatea lucrărilor de specialitate (1, 2), arată că insecta iernează în stadiul de adult, în interiorul galeriilor în care s-a format, în timpul perioadei de vegetaţie înmulţindu-se într-o singură generaţie. În primăvară (aprilie—mai) insectele adulte ies la lumina zilei, se răspîndesc pe trunchiul şi ramurile pomilor şi se hrănesc cu muguri. După împerechere, femelele săpînd galerii caracteristice, depun cîte 45—50 ouă fiecare, în galeriile de ordin secundar, roase tot de către ele. Larvele apărute se hrănesc cu miceliul ciupercii *Monilia* sau *Ambrosia candida,* vehiculată de insecta însăşi şi care dezvoltîndu-se, căptuşeşte pereţii galeriilor roase. După cercetările lui Stark (12), ciuperca se dezvoltă numai în galeriile roase la pomii în vegetaţie.

În ce priveşte speciile de carii de scoarţă (Eccoptogaster rugulosus şi E. mali), acestea iernează ca larve ajunse la completa dezvoltare. Insectele adulte apar în cursul lunii aprilie, fiind capabile să se înmulţească în cursul perioadei de vegetaţie în 1—2 generaţii (fig. 1).

MAGDALENA GEORGESCU și N. POMOHACI

VINIFICAȚIA ȘI VINURILE ÎN R. S. S. GRUZINĂ ● *Wine making and wines of the Soviet Socialist Republic of Gruziya* ● *Vinification et vins dans la R. S. S. Gruse* ● *Виноделие и вина в Грузинской ССР* 37

POMICULTURA

P. POPA

STABILIREA METODELOR ȘI A TIMPULUI OPTIM PENTRU ALTOIREA NUCULUI ● *Establishing the methods and optimum time for walnut grafting* ● *Etablissement des méthodes et du temps optime pour le greffage du noyer* ● *Установление методов и оптимального времени для прививки ореха* 42

I. BOTU

ASPECTE CU PRIVIRE LA APLICAREA TĂIERII DE RODIRE A PIERSICULUI ● *Aspects of bearing pruning of peach tree* ● *Aspects concernant l'appliquation de la taille fruitière du pêcher* ● *Аспекты в связи с применением плодоносящих обревок персика* 48

FLORICULTURA

ST. WAGNER

ROLUL INTERFERTILITĂȚII ÎN ALEGEREA GENITORILOR LA HIBRI-

1969

NEGRITU, V.

Aspecte noi privind combaterea mucegaiului cenușiu (Botrytis fuckeliana Buchw.) la Struguri. [New aspects in the control of B. fuckeliana of Grapevine.]

Revta Hortic. Vitic., 18 (4):3-13.

Microfilm no. 15622

VICTORIA ȘUTA și AL. V. ALEXANDRI

CERCETĂRI ASUPRA COMBATERII PE CALE CHIMICĂ A RAPĂNULUI PĂRULUI (ENDOSTIGME PIRINA — ADERH. SYD.) ÎN ROMÂNIA ● *Research on chemical control of Endostigme pirina Aderh — Syd. in Romania* ● *Recherches sur le combat par voie chimique de la gale (Endostigme pirina — Aderh — Syd) en Roumanie* ● *Исследования химической борьбы против парши (Endostigme pirina-Aderh-Syd) в Румынии* 75

V. LEMENI

BOLILE ȘI DĂUNĂTORII CARE ATACĂ GERBERA ȘI COMBATEREA LOR ● *Pests and diseases of Gerbera and their control* ● *Maladies et nuisibles Gerbera et la lutte contre eux* ● *Болезни и вредители жерберы и борьба с ними* . . 82

CRONICĂ ● CRONICLE ● CRONIQUE ● ХРОНИКА

Aspecte noi privind combaterea mucegaiului cenuşiu (**Botrytis fuckeliana Buchw.**) la struguri

Ing. V. NEGRITU
Departamentul Întreprinderilor Agricole de Stat

De cîţiva ani viile din ţara noastră, în special cele pentru struguri de masă, plătesc un greu tribut datorită atacului produs de Botrytis fuckeliana Buchw., care provoacă mucegaiul cenuşiu al strugurilor.

Se poate afirma că în ultimii trei ani, mucegaiul cenuşiu a produs mai mari pagube în vii decît cele cauzate de mană şi oidium la un loc, devenind cea mai dăunătoare boală criptogamică a plantaţiilor viticole. Situaţia — aşa cum reiese din publicaţiile de specialitate — este asemănătoare şi în alte ţări viticole europene : Franţa, R. F. a Germaniei, U.R.S.S., Bulgaria ş.a.

În cele ce urmează ne propunem să trecem în revistă condiţiile noi intervenite în cultura vieţi de vie, factorii care influenţează intensificarea atacului de mucegai cenuşiu, perfecţionarea metodelor de luptă contra bolilor şi dăunătorilor în general şi a lui Botrytis fuckeliana în special, pentru a putea găsi explicaţiile necesare înţelegerii acestor fenomene. Aceasta va permite luarea de măsuri adecvate pentru prevenirea, limitarea şi combaterea atacului de mucegai cenuşiu.

Botrytis fuckeliana este o ciupercă foarte răspîndită în natură. Este interesant de reţinut că ciuperca se dezvoltă la fel de bine pe ţesuturi moarte — saprofit — ca şi pe ţesuturi vii — parazit. Este polifagă, natura gazdelor sale vegetale fiind foarte variată.

Datorită acestor particularităţi, există în plantaţiile viticole surse permanente şi numeroase de infecţie a organelor verzi a viţei de vie, şi în special a strugurilor.

Pe organele atacate apar pete cenuşii, care sînt acoperite de conidiofori, ce poartă conidii brune. Germinarea conidiilor se face în apă sau medii nutritive, dacă sînt condiţii de temperatură. Filamentul germinativ al conidiei poate pătrunde în organele vii a plantelor prin eventualele răni ale acestora, sau direct prin suprafeţele lor intacte.

Sînt de reţinut două caracteristici esenţiale a miceliului ciupercii :

— capacitatea sa mare de rezistenţă, în ţesuturile moarte, în condiţii grele de iarnă, precum şi lunga sa viabilitate, în condiţiile existenţei unei umidităţi convenabile ;

— aptitudinea miceliului de a se extinde la suprafaţa organelor vii sau a ţesuturilor moarte atacate, care se menţin timp îndelungat dacă atmosfera este umedă, trecînd foarte uşor de pe un suport pe altul, jucînd în acest fel un rol foarte important în viaţa ciupercii precum şi de extindere a atacului asupra strugurilor.

Indiferent de tipul de infecţie — prin filamentul germinativ al conidiilor sau prin hife ale miceliului, condiţiile de mediu cerute, ca aceasta să aibă loc sînt aceleaşi.

Fie că infecţia se produce prin intermediul rănilor de pe organele verzi ale viei, sau direct prin suprafaţa intactă a lor, ca ea să se producă,

țesuturile respective trebuie să fie umede permanent, pe toată durata cît aceasta se produce.

Timpul necesar pentru infecție, în funcție de temperatură și natura țesutului atacat, variază între 12 și 30 de ore. Umiditatea necesară producerii infecției, poate proveni din precipitațiile atmosferice sau din scurgerile rănilor de pe organele atacate ale viei. Este interesant de subliniat, că mediul lichid necesar producerii infecției, trebuie să fie liniștit, agitarea lui fiind nocivă ciupercii.

Pentru ca ciuperca să poată emite miceliu exterior și conidii — primul necesar contaminărilor apropiate, conidiile contaminărilor de la distanță — este necesar ca umiditatea aerului să fie peste 80%.

CUM ATACĂ BOTRYTIS FUCKELIANA BUCHW. VIȚA DE VIE

Ciuperca poate ataca toate organele erbacee ale viței de vie, inclusiv boabele în cursul coacerii și chiar la supracoacere. În consecință epocile în care se produce atacul sînt numeroase, începînd din primăvară și întinzîndu-se pînă toamna tîrziu.

Primăvara și vara, ciuperca atacă frunzele, lăstarii și inflorescențele. Infecția se face de regulă prin rănile de diversă natură existente pe organele erbacee a viei (produse de insecte, traumatisme, cicatricile glandelor perlante etc.), acolo unde stagnează apa din precipitații timp mai îndelungat. Pentru ca infecția să se producă, sînt necesare însă și condiții de temperatură și umiditate aeriană convenabile.

Infecția poate fi favorizată de factorii morfologici ai plantei, sistemul de susținere a viei, lucrările culturale ce se aplică viei, sistem de fertilizare, biostimulatori etc., precum și de caracteristicile fiziologice ale organelor atacate (concentrația sucului celular, raportul dintre acizii organici din sucul celular, raportul conținutului în azot etc.).

Atacul pe organele verzi ale plantei nu produce în general pagube însemnate — de altfel nici nu s-au prea urmărit și studiat aceste pagube, fapt pentru care nici nu se obișnuiesc tratamente de combatere în această fază. Aceste atacuri însă măresc considerabil sursele de contaminare pentru strugurii în coacere.

Atacul pe struguri însă, produce pagube mari care în anii cu condiții deosebit de favorabile ciupercii, pot să fie chiar catastrofale, putînd merge pînă la compromiterea totală a recoltei.

În acești ani, putregaiul cenușiu se situează înaintea manei și oidiumului, privind importanța pagubelor pricinuite de atacul lor.

Strugurii pot fi atacați atît în stare erbacee, cît și în faza de coacere și chiar la supracoacere. Pătrunderea infecțiilor se face de regulă prin diversele leziuni ce se găsesc pe diferitele părți ale strugurilor, cum sînt cele provenite de la căderea componentelor care alcătuiesc florile sau cele produse de înțepăturile insectelor sau de loviturile de grindină etc. Atacul în acest stadiu de dezvoltare a strugurilor nu este de natură să pună probleme viticultorilor, el contribuind însă la creșterea surselor de infecție pentru strugurii în faza de coacere. În acest caz, infecția se face de regulă prin rănile deschise de pe boabe, răni ce pot fi provocate de insecte (viespi etc.), larvele de cochilis și eudemis, păsări, grindină, oidium, crăparea boabelor din exces de umezeală, diverse traumatisme etc. Pot constitui căi de

infecţie şi necrozele de pe boabe, produse în cursul vegetaţiei de diferite cauze (insolaţie, arsuri date de produsele fitofarmaceutice etc.). După cum am văzut mai sus, infecţia se poate face şi direct, prin pieliţa intactă a boabelor. Acest tip de infecţie este însă destul de redus, deoarece după cum am văzut, pentru a se putea produce, necesită prezenţa continuă a apei pe boabe timp îndelungat pînă la 30 de ore.

Pe struguri, Botrytis fuckeliana provoacă două tipuri de mucegai : mucegaiul cenuşiu şi mucegaiul nobil.

Mucegaiul cenuşiu se manifestă pe struguri, cînd atacul se produce de la intrarea lor în pîrgă pînă la maturitatea fiziologică şi apoi în cursul supracoacerii. Acest tip de mucegai este foarte păgubitor, atît prin diminuarea cantitativă cît şi prin deprecierea calitativă a recoltei.

Cînd strugurii sînt complet copţi şi au un conţinut în zahăr de minim 200 g/l, concentraţia mare de zahăr din pulpă devine nefavorabilă dezvoltării miceliului mucegaiului cenuşiu. În acest caz, ciuperca atacă doar pieliţa boabelor, pe care o distruge. În urma acestui atac pieliţa devine permeabilă şi permite evaporarea cu uşurinţă a apei din boabe. Datorită acestui mecanism, concentraţia în zahăr creşte foarte mult, strugurii devenind o materie primă excelentă pentru obţinerea de vinuri licoroase naturale, cu calităţi gustative excepţionale (Tokay, Sauvignon, Cotnar, Murfatlar). Acest tip de mucegai se numeşte „mucegaiul nobil“ şi în anumite podgorii este de dorit. Prin urmare, pentru ca atacul de mucegai nobil să se producă, sînt necesare două condiţii esenţiale : concentraţia în zahăr peste 200 g/l şi o consistenţă apoasă a pulpei, ca să se poată produce evaporarea apei (strugurii au pulpa cărnoasă, consistentă, nefiind atacaţi de mucegaiul nobil).

Este de remarcat faptul că zahărul, la începutul coacerii este un factor favorizant al atacului de mucegai cenuşiu, devenind apoi defavorizant atunci cînd concentraţia depăşeşte 200 g/l, iar la supracoacere, devine din nou favorizant. Se poate deci deduce că nu creşterea concentraţiei în zahăr este factorul favorizant sau defavorizant al atacului de mucegai, ci schimbările fiziologice care au loc în acele perioade în funcţiunile boabelor.

FACTORII CARE FAVORIZEAZĂ ATACUL

Volumul pierderilor cauzate de mucegaiul cenuşiu sînt în raport direct cu condiţiile favorizante ale atacului. Dintre acestea, se impun atenţiei, ca mai importante următoarele :

— **Regimul pluviometric.** Cu cît ploile sînt mai multe şi mai abundente în perioada de coacere a strugurilor cu atît atacul de mucegai va fi mai puternic, iar pagubele mai mari.

— **Durata de stagnare a apei.** Prezenţa apei pe organele verzi ale viei este o condiţie esenţială a infecţiei de mucegai. Aşa după cum am văzut mai sus, ca infecţia să aibă loc, stagnarea trebuie să fie de lungă durată, de la 15—20 ore în sus, iar apa trebuie să fie în stare liniştită. Stagnarea este influenţată de relieful plantaţiei (pe pante, unde sînt curenţi mai mari, stagnarea este mai mică), de înălţimea butucilor (la nivelul solului higroscopicitatea este mai mare), de bogăţia sistemului foliar, de orientarea rîndurilor şi de distanţele de plantare, de portul natural al soiurilor etc.

— **Însuşirile morfologice.** Compactitatea strugurilor, consistenţa pulpei, grosimea pieliţei boabelor, mărimea boabelor etc., sînt tot atîţia factori care pot contribui la declanşarea şi extinderea atacului.

— **Însuşirile fiziologice.** Din datele culese pînă în prezent, se pare că aceste însuşiri sînt mult mai importante în favorizarea atacului de mucegai ca însuşirile morfologice. Concentraţia sucului celular este un factor foarte important : cu cît concentraţia sucului celular este mai mare, cu atît atacul este mai mic (excepţie făcînd concentraţia zaharurilor, care aşa cum am văzut, o dată cu creşterea ei, creşte şi favorabilitatea atacului de mucegai). Acest fenomen ne explică cauza pentru care viile mai puţin viguroase, sînt în general mai puţin atacate de mucegai.

Nu sînt străine de sensibilizarea plantei la atac nici structura interioară a ţesuturilor organelor atacate, echilibrul între compuşii chimici ai sucului celular, raportul de azot din aceste ţesuturi, funcţiile biochimice a ţesuturilor etc.

— **Fertilizarea plantaţiilor.** Se consideră că unul din factorii esenţiali responsabili de crearea unor premise de mare favorabilitate a atacului de mucegai, este excesul de azot în sol. O fertilizare bogată cu azot favorizează o creştere luxuriantă, frunziş bogat, ţesuturi mai puţin dense, cu o concentraţie mică a sucului celular. Toate acestea sînt factori favorizanţi ai atacului. Mai mult, excesul de azot produce şi un dezechilibru al raportului de N în sucul celular, dezechilibru ce este considerat ca unul din factorii majori ai sensibilizării plantelor la atacul de mucegai cenuşiu. Fertilizarea excesivă cu azot este cu atît mai dăunătoare, cu cît este făcută unilateral, nerespectîndu-se raportul optim între elementele nutritive ce se administrează plantaţiilor.

— **Biostimulatorii de creştere.** Substanţele cu acţiune biostimulatorie, intervin activ în viaţa intimă a plantelor, putînd produce modificări esenţiale în morfologia şi biologia lor. Ori, aşa cum am văzut, unele din aceste modificări pot fi favorizante sau defavorizante pentru atacul de mucegai. Aşa de exemplu, sub acţiunea Giberelinei, strugurii se alungesc mult, făcîndu-i mai puţin compacţi, făcîndu-i mai puţin sensibil la atac.

Alţi biostimulatori, ca de altfel şi unele insectofungicide de sinteză, favorizează creşterea şi implicit şi atacul de mucegai (cazul produsului Zineb, utilizat pentru combaterea manei, care acţionează şi cu biostimulator, favorizînd creşterea).

Influenţa noilor produse fitofarmaceutice utilizate în combaterea dăunătorilor nu este încă suficient studiată în ceea ce priveşte acţiunea lor asupra biologiei plantelor. Se cunosc totuşi destule cazuri, cînd datorită acţiunii acestor produse asupra biologiei plantei, o dată cu combaterea dăunătorilor pentru care sînt utilizate, produc sensibilizarea plantelor la atacul altor dăunători (cazul produsului Zineb, care o dată cu combaterea manei, sensibilizează foarte mult planta la atacul de acarieni).

— **Însuşirile specifice de soi.** Se ştie că soiurile precoce sînt mai puţin atacate ca cele tardive, după cum soiurile ce acumulează zahăr mai mult, sînt mai puţin sensibile ca cele ce acumulează mai puţin. Soiurile cu boabe cu pulpa cărnoasă sînt mai sensibile la atac, ca efect al unui conţinut mai mic în zahăr şi a predispoziţiei lor pentru crăpare. Soiurile cu strugurii compacţi sînt mai susceptibile atacului, ca cele cu struguri răsfiraţi,

aerisiţi. Vigoare mare naturală a butucilor, este de asemenea un factor favorizant al atacului.

În ceea ce priveşte grosimea pieliţei boabelor, precum şi grosimea stratului cu pruină, nu constituie un obstacol în calea atacului. Deşi mecanismul pătrunderii filamentului ciupercii prin pieliţa intactă a boabelor nu este binecunoscut, infecţia este facilitată de o enzimă, care atacă pereţii celulari. Din această cauză important nu este grosimea pieliţei, ci compoziţia pereţilor celulari ce o compun. Elucidarea acestei probleme ar putea explica rezistenţa naturală la atacul de mucegai a unor soiuri de hibrizi producători direcţi, ca Isabelle, Noahè ş.a. şi ar putea sta la baza lucrărilor de ameliorare, în vederea creării de soiuri rezistente la atacul de mucegai.

— **Atacul de dăunători.** Leziunile produse de atacul insectelor, — larvele de cochilis şi endemis, viespi etc. — şi a bolilor criptogamice — oidium — sînt tot atîtea portiţe de atac direct a mucegaiului pe struguri. Cu cît aceste leziuni vor fi mai multe, cu atît condiţiile de atac cresc, cunoscut fiind că pe strugurii în coacere leziunile prin scurgerile lor asigură şi umezeala necesară infecţiei, chiar în absenţa precipitaţiilor.

— **Traumatismele accidentale.** Şi leziunile produse de grindină, insolaţie, loviri cu uneltele sau de sol, crăparea boabelor în urma ploilor sau irigării neraţionale etc., au acelaşi rod în favorizarea atacului de mucegai, ca şi leziunile produse de dăunători.

— **Lucrările culturale.** Prin executarea sau neexecutarea lucrărilor de întreţinere a solului şi asupra butucului de vie, se poate favoriza sau defavoriza atacul de mucegai. Este ştiut că prezenţa numeroasă a buruienilor, neînlăturarea lăstarilor copililor şi chiar a o parte din frunzele care nu sînt utile producţiei, duc la crearea şi menţinerea unui mediu de higroscopicitate ridicată, a stagnării îndelungate a apelor din precipitaţii, creînd astfel condiţii bune pentru infecţie şi extinderea atacului.

LUPTA CONTRA MUCEGAIULUI CENUŞIU

Datorită faptului că ciuperca nu produce pagube mari decît în anii favorabili atacului, iar tratamentele specifice aveau eficacitate redusă, combaterea mucegaiului cenuşiu a fost neglijată, rămînînd în exclusivitate pe seama zemei bordeleze, a cărei concentraţii în SO_4Cu era de obicei între 1 şi 2%.

Cum însă în ultima vreme, datorită condiţiilor noi create în plantaţiile viticole, atacul de mucegai a început să producă pagube din ce în ce mai mari, iar tehnica de combatere a făcut progrese însemnate, prin produse specifice noi şi eficace, combaterea mucegaiului devenind o problemă capitală pentru viticultori. S-au întreprins experienţe mai mult sau mai puţin intense în aproape toate ţările viticole din lume, rezultatele fiind încurajatoare.

Măsurile de luptă contra mucegaiului cenuşiu se pot împărţi în două categorii :

A — măsuri generale de prevenire şi diminuare a posibilităţilor de infecţie ;

B — tratamente chimice specifice.

A. Măsuri generale de prevenire şi diminuare a posibilităţilor de infecţii

Dintre principalele măsuri de prevenire şi diminuare a posibilităţilor de atac, cităm :

— Adaptarea unui sistem convenabil de cultură şi susţinere a plantaţiei, plecînd de la premisele cunoscute că : o vigoare mai moderată a butucilor, o aerisire mai bună a plantaţiei, o repartiţie mai echilibrată a organelor vegetative în spaţiu, o încărcătură mai raţională cu rod a butucilor, un nivel mai înalt al capului butucului de la sol, menţinerea plantaţiei fără buruieni, sînt tot atîţia factori care limitează posibilităţile de atac ale mucegaiului cenuşiu.

În acest sens va trebui bine studiată problema orientării rîndurilor, a distanţelor de plantare între rînduri şi pe rînd, sistemul de susţinere, numărul şi lungimea coardelor ce se lasă (încărcătura optimă a butucului), înălţimea optimă a capului butucului, executarea lucrărilor în verde, precum şi alte aspecte menite să evite cît mai mult posibil crearea de condiţii favorabile atacului de mucegai.

— Întocmirea unui plan raţional de fertilizare, plecînd de la potenţialul real de producţie a plantaţiei, în funcţie de soi, direcţie de producţie şi conţinutul solului în substanţe nutritive. Fertilizarea anuală a solului trebuie să asigure elementele nutritive în vederea realizării producţiei planificate, atît cantitativ şi calitativ. Forţarea creşterii producţiei prin fertilizări unilaterale cu azot, sau nerespectarea raportului optim dintre elementele nutritive necesare viţei de vie, duce la crearea de dezechilibru în vegetaţie, creşteri luxuriante cu ţesuturi laxe, schimbarea echilibrului conţinutului sucului celular etc., care pe lîngă că pot duce la o scădere a calităţii producţiei, creează şi condiţii favorabile atacului de mucegai.

— Eliminarea cauzelor rănirii boabelor, deşi infecţia se poate face şi direct, prin pieliţa intactă a boabelor, principalele posibilităţi de infecţie rămîn totuşi rănile deschise, cauzate de diferiţi agenţi. Pentru limitarea posibilităţilor de infecţie, este foarte important de a se lua măsuri energice pentru înlăturarea cauzelor care duc la rănirea boabelor. Dintre acestea cităm :

1. Combaterea eficientă a oidiumului, cochilisului şi eudemisului precum şi a altor boli şi dăunători. Datorită condiţiilor tehnico-materiale create, aceasta nu mai constituie o problemă, decît în măsura în care nu se execută la timp şi corect. Este interesantă în acest sens experienţa lui P. Hugliu, privind combaterea cochilisului şi eudemisului :

— la varianta tratată cu D.D.T. s-au înregistrat şi 9 struguri atacaţi de mucegai ;

— la varianta tratată cu esteri fosforici a înregistrat 6,8% struguri atacaţi de mucegai ;

— la varianta martor, unde nu s-a aplicat nici un tratament pentru combaterea cochilisului şi eudemisului, s-a înregistrat 75% struguri atacaţi de mucegai.

2. Prin lucrări de întreţinere executate la timp şi în bune condiţii tehnice, precum şi prin adaptarea unui sistem de susţinere corespunzător, se pot elimina în mare măsură cauzele rănirilor accidentale, ca cele produse de vînt, insolaţie, loviri cu uneltele de lucru sau de sol etc.

3. Prin întocmirea unui plan rațional de irigare, în plantațiile amenajate în acest scop, se va evita crăparea boabelor cauzată de excesul de apă în perioada coacerii.

— Introducerea în cultură a soiurilor cu rezistența naturală la atacul de mucegai. Din practică este știut că există soiuri care sînt sensibile la atacul de mucegai, precum și soiuri cu rezistență vădită la atac. În general, soiurile cu coacere precoce, care acumulează zahăr în cantități mai mari, cu vigoare moderată și cu strugurii mai aerisiți, mai răsfirați sînt mai rezistenți la atacul de mucegai, și deci vor fi preferate, bineînțeles dacă corespund și scopului de producție.

— Utilizarea biostimulatorilor de creștere și a produselor fitofarmaceutice care au acțiune biostimulatorie, se va face cu atenție și precauție, știind că prin influențarea biologiei plantelor pot duce la crearea de condiții favorizante pentru atacul de mucegai.

B. **Tratamente chimice**

Este ușor de înțeles că producătorii nu vor accepta cu ușurință acele măsuri de combatere care tind să diminueze vigoarea butucului și implicit a producței pe unitatea de suprafață, fiind mult mai interesați în combaterea directă a mucegaiului, prin tratamente chimice.

Primele încercări de combatere chimică a mucegaiului cenușiu s-au făcut prin tratamente cu SO_4Cu (zeamă bordeleză). Ionii de cupru s-au dovedit capabili să împiedice germinarea conidiilor de mucegai în medii lichide, însă la concentrații mult mai mari ca cele necesare combaterii manei.

Adăugînd la aceasta și faptul că în timp ce pentru combaterea manei, redistribuirea ionilor de cupru de pe organele plantei de către apa din precipitații este destul de eficientă pentru oprirea infecției mucegaiului cenușiu. Această operație fiind nesatisfăcătoare, ne dăm ușor seama de cauzele slabei eficacități a zemei bordeleze, în combaterea mucegaiului cenușiu.

Grație fungicidelor de sinteză și progresului în construirea aparatelor de stropit, combaterea mucegaiului cenușiu a fost reluată în ultimii ani cu perspective noi.

Deși din punct de vedere științific problema are încă multe aspecte necunoscute. Din punct de vedere practic există deja un volum destul de mare de experimentări, a căror rezultate au contribuit la realizarea unor progrese apreciabile în direcția combaterii chimice a mucegaiului.

În urma multiplelor experimentări, s-a constatat că au o eficacitate mulțumitoare, următoarele fungicide: Euparen (Dichlofluanid, Phaltan (Phaltocid), Orthophaltan, T.M.T.D. (Thiram) 80%, Difolatan (Phalcid), Captan, Ls. 63—319 (Thiabendazol), G. 84 (Dachonil), Basfungin.

Toate aceste produse au manifestat acțiune antibotritică, dar în condiții de mediu diferite rezultatele nu sînt întotdeauna în concordanță.

Euparenul, se recomandă să fie folosit în doză de 4 kg/ha prin pulverizare pneumatică, dînd în mod constant rezultate destul de bune antibotritice. Este considerat cel mai eficace produs contra mucegaiului.

Acest preparat nu combate însă mana decît în foarte mică măsură. Este ușor fitotoxic și cu acțiune slabă ca antiferment. Se impune ca cel mai bun produs antibotritic pentru tratamentele specifice.

Phaltanul, este utilizat în doze de 4 kg/ha, dă rezultate destul de bune în combaterea mucegaiului. Nu este fitotoxic şi combate bine şi mana. Este însă puternic antiferment. Este considerat ca unul din cele mai bune produse antibotritice.

Pe strugurii de masă lasă însă pete, care le strică aspectul comercial.

Orthophaltanul, este un produs care dă rezultate bune în combaterea manei şi mulţumitoare în combaterea mucegaiului. Se întrebuinţează în doză de 0,2—0,3%. Nu este fitotoxic şi nu are acţiune evidentă antiferment.

T.M.T.D. — **80%** (Thiram sau Tiuram) utilizat în doze de 4 kg/ha, produs comercial la o stropire, a dat rezultate mulţumitoare antibotritis. Nu combate însă mana, nefiind fitotoxic şi nu are acţiune antiferment. Este compatibil cu sulf muiabil, Captan şi pesticide.

Difolatanul (80% Pholcid), dă în general rezultate bune antibotritis, deşi uneori contradictorii. În anumite condiţii este fitotoxic. Dă rezultate bune şi în combaterea manei, fiind produsul cu cea mai puternică acţiune antiferment.

Se utilizează în doze de 2,5 kg produs comercial la hectar, pentru o stropire. Lasă pete uşoare pe strugurii de masă.

Captanul (Ortocid 50 sau Permidin), se utilizează în doză de 0,2% în combaterea manei. Are eficacitate mulţumitoare şi contra mucegaiului. Nu este fitotoxic, dar are acţiune antiferment, fiind şi biostimulator, favorizînd creşterea. Este compatibil cu sulful muiabil şi toate insecticidele utilizate în vii. Nu este compatibil cu zeama bordeleză şi sulfocalcică. Lasă pete pe strugurii de masă.

Ls 63-319 (Thiobendazol), este un produs nou care experimentat în doză de 4 kg/ha produs comercial la o stropire, a dat rezultate mulţumitoare antibotritis. Din rezultatele înregistrate, se pare a fi un produs de perspectivă.

G. 84 (Daconil), este de asemenea un produs nou care în experimentările făcute, s-a impus atenţiei, cu rezultate antibotritis destul de bune.

Basfunginul, produs de firma BASF din R.F. a Germaniei, fiind utilizat experimental în 1968 şi în ţara noastră. Utilizat în doză de 0,20—0,25% produs comercial, în unele experienţe a dat rezultate satisfăcătoare.

Desigur, gama produselor antibotritis experimentale este mult mai mare, dar din lipsa unor experimentări temeinice, nu pot fi luate încă în considerare.

Cînd trebuie aplicate tratamentele

Pentru a lupta cu succes contra atacului de mucegai pe strugurii în coacere, s-a constatat că este absolut necesar ca tratamentele să înceapă de timpuriu, de la înfloritul viei.

În urma unor intense experimentări, cercetătorii francezi au ajuns la o formulă de combatere cu patru tratamente. Acestea urmează a se executa astfel :

— primul tratament în timpul înfloritului sau imediat după înflorire ;

— al doilea tratament, la 15—18 zile după primul ;

— al treilea tratament la 15—18 zile după al doilea ;

— ultimul tratament urmează a fi făcut la 10—20 zile după începutul coacerii în pîrgă.

Bineînţeles că această schemă nu trebuie privită ca un tablou fix ; ea trebuie adoptată condiţiilor locale de climă şi a sensibilităţii soiurilor cultivate.

În podgoriile sudice şi la soiurile mai precoce, este probabil să fie suficiente doar două tratamente înaintea închiderii strugurilor şi un tratament după începerea coacerii.

Din contra, în podgoriile mai nordice, unde culesul se face în octombrie-noiembrie, iar toamnele sînt ploioase, un singur tratament în perioada coacerii va fi în mod sigur insuficient. În aceste cazuri vor fi necesare 2 şi chiar 3 tratamente contra mucegaiului în această perioadă.

În ceea ce priveşte modul de aplicare a tratamentelor antibotritis, din experimentările făcute, cele mai bune rezultate s-au obţinut cînd acestea s-au executat prin pulverizare cu pulverizatoare pneumatice, cu debit mic de soluţie şi consum mare de aer, dirijate manual.

Prin acest sistem se asigură o distribuire bună a produsului pe organele butucului şi în special pe ciorchini, condiţie esenţială pentru asigurarea unei bune protecţii contra infecţiilor de mucegai.

Subliniem că ineficacitatea unor produse antibotritis experimentate se datoreşte în cea mai mare măsură nedistribuirii produsului pe toate organele viţei de vie şi în special pe ciorchini, ca urmare a utilizării unor sisteme de pulverizare neadecvate.

Combaterea mucegaiului prin prăfuirea produselor, a dat întotdeauna rezultate inferioare pulverizării.

În privinţa dozelor ce trebuie aplicate, ele trebuie bine analizate, trebuind să răspundă atît problemelor de eficacitate, cît şi celor de cost. Trebuie aplicată relaţia doză-eficacitate, în funcţie de gradul de infecţie (la atacuri puternice se vor administra doze mai mari şi invers).

EFECTELE TRATAMENTELOR

La toate experimentările şi la toate produsele, nu s-a obţinut o combatere completă a mucegaiului, înregistrîndu-se un număr mai mare sau mai mic de struguri atacaţi.

Ceea ce trebuie însă reţinut, este faptul că prin aceste tratamente s-a reuşit să se protejeze o importantă parte a recoltei pe o perioadă limitată de timp. Prin aceasta, viticultorul are la dispoziţie o perioadă mai lungă pentru efectuarea culesului ceea ce-i va permite în anii cu atac puternic de mucegai, să recolteze fără pierderi prea mari, atît cantitative cît şi calitative.

Toate produsele experimentate pînă în prezent pătează strugurii de masă, stricîndu-le aspectul comercial. Deoarece ultimul tratament se face cu 15—20 zile înaintea culesului, spălarea petelor de apa ploilor este practic imposibilă, cercetătorii sînt în căutarea unui produs care să nu păteze.

Trebuie de reţinut şi faptul că acţiunea antiferment, care la unele produse antibotritis este puternică, poate crea dificultăţi mari la vinificare, prin întîrzierea intrării în fermentare.

Această întîrziere poate fi uneori de 8—10 zile, ceea ce duce la creşterea considerabilă a necesarului de spaţiu pentru fermentare.

Consecințele igienice ce pot surveni ca urmare a consumului de struguri tratați cu produse antibotritis, precum și eventuala influență a acestor produse asupra calității vinurilor obținute din struguri tratați, nu se cunosc încă, nefiind cercetate.

În ceea ce privește fitotoxicitatea manifestată de unele produse antibotritis, ea s-a înregistrat pe toate organele verzi ale plantei. Se remarcă soiuri sensibile și soiuri rezistente.

Sînt de mare importanță leziunile produse pe boabe la strugurii de masă, stricîndu-le aspectul comercial. Trebuie de reținut că boabele tinere sînt mai sensibile ca cele ajunse în pîrgă, iar strugurii umbriți mai sensibili ca cei însoriți. De asemenea fititoxicitatea crește în raport direct cu durata stagnării soluției pe organele plantei. Aceste date sînt foarte importante la alegerea produsului ce se va întrebuința pentru combaterea mucegaiului.

Atunci cînd se fac tratamente mixte pentru combaterea manei și mucegaiului, se va ține seama de următoarele principii :

— produsele antibotritis trebuie date în mod obligatoriu direct pe struguri ;

— concentrațiile necesare combaterii mucegaiului, sînt de regulă mai mari ca cele necesare combaterii manei ;

— dacă se întrebuințează aparate de stropit cu organe de distribuție fixe, acestea se vor orienta cît mai bine, pentru a distribui soluția direct pe struguri ;

— presiunea aparatului de stropit se va regla de așa manieră, pentru ca pătrunderea soluției în interiorul butucului pe struguri, să fie cît mai activă ;

— tratamentele cu Euparen, Orthophaltan, și Difolatan se vor face la 15 zile după tratamentul cu zeamă bordeleză contra manei, aceste produse fiind incompatibile cu reacția alcalină a varului.

CONCLUZII

1. Botrytis fuckeliana Buchw. care produce mucegaiul cenușiu, este o ciupercă foarte răspîndită, polifagă, trăind pe organele viței de vie atît ca parazit, cît și ca saprofit. Produce infecția și se răspîndește prin conidii și prin fragmente de miceliu. Produce pagube foarte mari cînd atacă strugurii în coacere.

În anumite condiții de concentrație de zahăr, atacă sub formă de mucegai nobil, cînd contribuie la creșterea calității vinurilor obținute din strugurii atacați.

2. Toate fungicidele experimentate nu au asigurat o combatere completă a mucegaiului. Se remarcă cu efectele bune Euparenul, Phaltanul, Orthophaltanul și T.M.T.D.-ul.

Unele din aceste produse combat foarte bine și mana. Altele prezintă fenomene de fitotoxicitate și acțiune inhibitoare pentru fermenți.

Zeama bordeleză în concentrații de peste 2% SO_4Cu, are și acțiune antibotritis.

3. Pulverizatoarele pneumatice, cu debit mic de soluție și presiune mare, dirijate manual, dau cele mai bune rezultate în combaterea mucegaiului. Prăfuirea dă rezultate mai slabe ca pulverizarea.

Tratamentele antibotritis se pot suprapune cu cele contra manei, dacă se întrebuinţează produse cu dublă eficacitate sau combinaţii de mai multe produse, cu condiţia să fie compatibile şi să fie bine distribuite direct pe struguri. După începerea pîrgii însă se vor aplica 1—3 tratamente special antibotritis.

5. Măsurile generale de prevenire şi limitare a posibilităţilor de infecţie, trebuie să fie introduse printr-un plan de cultură şi susţinere a viţei de vie, bine pus la punct.

6. Pagubele produse de mucegaiul cenuşiu trebuie privite la importanţa lor reală, în funcţie de condiţiile pedoclimatice ale fiecărei podgorii, ele impunînd măsuri adecvate de combatere, care să satisfacă atît aspectele de eficienţă tehnică, cît şi cele economice.

Bibliografie

Branas J. — Cu privire la putrezirea cenuşie a strugurilor. Le progres agricole et viticole nr. 13/1967

Branas J. — Lupta contra putrezirii cenuşii a strugurilor. Le progres agricole et viticole nr. 14/1967

Sandu Ville C. — Contribuţii la cunoaşterea biologiei ciupercii Botrytiona fuckeliana, parazită pe viţa de vie şi combaterea ei. Fascicolul 2 — Acad. R.P.R. — Filiala Iaşi, 1962

Levin Gh. — Atacul putregaiului cenuşiu al strugurilor în podgoria Dealul Mare. Analele I.C.V.V., vol. I, 1968

Uanev S. — Factorii meteorologici şi putrezirea cenuşie a strugurilor. Gradin, Loz. Nauca U.I.-Sofia

3. *Alexandru V. Al.* şi *Lucescu S.* — Realizări noi în combaterea buruienilor pe cale chimică, Ed. Inst. de doc. tehnică, Bucureşti, 1964.
4. *Cirbadjuska B.* şi *Atanasov A.* — Combaterea chimică a buruienilor în culturile de piersici şi legume. Ovosciarstvo nr. 12/XII.1963.
5. *Coman St.* — Folosirea erbicidelor în livezi. Revista de Horticultură şi Viticultură nr. 4/1967.
6. *Eisenbarth H. J.* — Posibilitatea combaterii chimice a buruienilor din vii. Weinberg und Keller, 12, nr. 3, martie 1965.
7. *Michel H. G.* — Combaterea buruienilor în pomicultură. Der Obstbau, 84, nr. 4, mai, 1965.
8. *Nedelcev N.* — Zameneanc na obragotkite ne procivota lozeata s herbitidî. In Brandinarska i lazarska nauka, R.P. Bulgaria, 2, nr. 4, 1965.
9. *Şarpe N. şi colab.* — Combaterea buruienilor cu ajutorul erbicidelor. Referate susţinute la consfătuirea internaţională de la Bucureşti, iunie, 1962.

CHEMICAL WEED CONTROL IN ORCHARDS

The study conducted by the author permitted him to come to the conclusion that :

1969

SUTA, VICTORIA & ALEXANDRI, A.V.

Cercetări asupra combaterii pe cale chimică a rapănului părului (Endostigme pirina Aderh Syd.) în România. [Investigations on the chemical control of Venturia pirina in Rumania.]

Revtă Hortic. Vitic., 18 (4):75-81. [4 fig., 3 tables. Stat. exp. Pomic. Voinesti.]

Microfilm no. 15623

ХИМИЧЕСКАЯ БОРЬБА С СОРНЯКАМИ В ПЛОДОВЫХ САДАХ

В результате исследований автор пришел к выводу, что эффективность гербицидов тесно связана с влажностью почвы, дожди или орошения способствуя равномерному перемещению их на уровень корней сорняков. Атразин и дозе 10 кг/га, применяемый до всхода сорняков, и главным образом А-1089 (30 кг/га) применяемый до и после всхода сорняков, дали самые лучшие результаты.

Гербициды на основе триазин уничтожили большое количество однолетних сорняков, но не уничтожили двудольные виды.

Cercetări asupra combaterii pe cale chimică a rapănului părului (Endostigme pirina-Aderh Syd.) în România*)

Dr. ing. VICTORIA ŞUTA
Staţiunea experimentală pomicolă Voineşti
Dr. AL. V. ALEXANDRI
Institutul de cercetări pentru protecţia plantelor

Rapănul părului (Endostigme pirina — Aderh. Syd.) constituie o problemă îndeosebi pentru soiurile de păr cu coacere tîrzie din ţara noastră, deoarece poate provoca pierderi calitative şi cantitative evaluate la 50—100% din recoltă.

Numeroşi cercetători din Europa au ajuns la concluzia, că rapănul este una dintre cele mai periculoase boli ale părului (1), (2), (3), (5), (6), (11), (12), iar combaterea ei nu poate fi rezolvată decît prin cercetări aprofundate, asupra biologiei agentului fitopatogen, în fiecare zonă de cultură a părului.

În condiţiile pedoclimatice din România, s-au făcut o serie de cercetări privind biologia şi combaterea rapănului la păr (8), (9), cercetări îndreptate şi asupra rezistenţei soiurilor de păr faţă de atacul acestui agent patogen (8), (10) şi stabilirii momentului optim de aplicare a tratamentelor.

Experimentările au fost făcute la Staţiunea experimentală pomicolă Voineşti, judeţul Dîmboviţa, care este situată în zona dealurilor subcarpatice, la altitudinea de 550 m. S-a folosit soiul de păr Curé, care s-a dovedit a fi printre cele mai sensibile la rapăn (10).

Tratamentele s-au aplicat la avertizare, folosindu-se aceeaşi metodă ca şi în cazul combaterii rapănului la măr (7) şi în care s-a luat ca bază maturarea periteciilor, proiectarea ascosporilor, importanţa condiilor de vară, corelate cu observaţiile meteorologice şi observaţiile fenologice. A fost încercată eficacitatea a 8 fungicide. O dată cu observaţiile asupra eficacităţii fungicidelor, s-au făcut observaţii şi asupra fitotoxicităţii acestora, folosindu-se aceeaşi metodă ca pentru atacul de rapăn.

S-au urmărit de asemenea şi pierderile cauzate de atacul de *Monilinia fructigena* (Aderh. et Ruhl.) Honey în depozit.

REZULTATELE OBŢINUTE

În anii 1965—1968 au fost condiţii favorabile dezvoltării acestei boli, determinate de frecvenţa precipitaţiilor şi de temperaturile favorabile.

Astfel, în anii 1965, 1966 şi 1968 în perioada aprilie—iunie, s-au înregistrat 41—43 zile cu precipitaţii, iar în anul 1967, 55 zile. Precipitaţiile căzute în aceşti ani au însumat între 168,6—265,7 mm. În luna iulie, în anii 1965—1966 şi 1968 s-au înregistrat 12—16 zile cu ploi, iar în anul 1967, au fost 6 zile.

---

*) Lucrarea a fost prezentată la cel de al VII-lea Congres de Protecţia Plantelor de la Viena din 1967, adăugîndu-se la studiul de faţă şi datele anului 1968.

Temperaturile medii zilnice au fost cuprinse în perioada de studiu între 6,4 şi 11,6°C, în luna aprilie, 12,6 şi 15,0°C, în luna mai şi 16,1 şi 19,8°C, în luna iunie.

Ţinînd seama de biologia agentului patogen, de fenologia plantelor şi de factorii meteorologici, au fost necesare anual 8—12 tratamente.

Condiţiile de climă variabile în aceşti ani, au făcut ca biologia agentului patogen să evolueze diferit de la an la an (tabelul 1). Astfel, începu-

Tabelul 1

Corelaţia dintre biologia agentului fitopatogen şi fenologia soiului de păr Guré

Anii	Elemente de biologia agentului fitopatogen şi de fenologia părului								
	Începutul maturării ascelor		Începutul proiectării ascosporilor		Sfîrşitul proiectării ascosporilor		Apariţia primelor pete de rapăn pe frunze		
	data obs.	% asce mature	data obs.	fazele mugurilor pe rod	data obs.	durata proiectării ascosporilor zile	data obs.	faza fenologică	durata perioadei de incubaţie nr. zile
1965	15 III	0,14	25 IV	Resfirarea inflorescenţei	11 VI	47	9 V	Începutul scuturării petalelor	14—16
1966	1 IV	0,28	26 IV	Înfoierea corolei	15 VI	50	10 V	Fructul cît bobul de mazăre	14—16
1967	1 IV	1,5	10 IV	Sfîrşitul desmuguritului	12 VI	61	10 V	Înfoierea corolei	17—20
1968	1 IV	0,42	7 IV	Resfirarea inflorescenţei	23 V	46	3 V	Fructul cît bobul de mazăre	14—22

tul proiectării ascosporilor în anii 1965 şi 1966 a avut loc la 25 şi 26 aprilie, iar în anii 1967 şi 1968 la 10 şi 7 aprilie, proiectarea ascosporilor durînd între 46—61 zile, sfîrşitul proiectării fiind între 23 mai şi 15 iunie.

Fenologia plantei a fost foarte diferită, în anii 1965 şi 1968, începutul proiectării ascosporilor a corespuns cu faza de răsfirare a inflorescenţei, în 1966 la înfoierea corolei, iar în 1967 la sfîrşitul desmuguritului.

Apariţia primelor pete de rapăn a avut loc între 3 şi 10 mai după 14 pînă la 20 zile de la contaminare, la faze de dezvoltare foarte diferite (ex. la înfoierea corolei, începutul scuturării petalelor şi cînd fructul a avut mărimea unui bob de mazăre).

Numărul tratamentelor aplicate la avertizare a fost 8 în 1965 dintre care 2 înainte de înflorit, 9 în 1966, dintre care 2 înainte de înflorit ; iar în 1968, s-au aplicat 12 tratamente dintre care 2 înainte de înflorit.

Tratamentele aplicate înainte de înflorit au privit infecţiile prin ascospori şi conidiile de primăvară, iar tratamentele aplicate după scuturarea petalelor au prevenit infecţiile secundare ce se fac prin conidii de vară, prin ultimii ascospori şi ultimele conidii din cele de rezistenţă de pe ramuri.

Cel mai mare pericol pentru infecţii îl constituie ascosporii care încep să se proiecteze obişnuit la sfîrşitul desmuguritului mugurilor florali, iar în unii ani la începutul înălţării, răsfirării inflorescenţei şi chiar la înfoierea corolei.

Tabelul 2

Eficacitatea tratamentelor pentru combaterea rapănului părului la Stațiunea Voinești

Denumirea fungicidului	Anii luați în studiu	Nr. tratamente	Observații asupra atacului de rapăn la: frunze		fructe		lăstari		Observații asupra fitotoxicității la: frunze		fructe		Producția	
			% atac	I	% atac	I	% atac	I	% frecv.	I	% frecv.	I	media pe variantă	semnificația
Martor fără tratament	1965	—	35,5	+±2	67,00	+±4	56,00	+±1	0	—	0,5	+	32,0	—
	1966	—	51,7	+±4	72,00	+±4	52,00	+±1	0	—	0,4	+	54,0	—
	1967	—	42,0	+±3	56,00	+±4	62,00	+±1	0	—	0,5	+	46,5	—
Zeamă bordeleză	1965	8	2,99	+	5,96	+	5,11	+	25,6	+	81,4	+±4	129,0	+++
	1966	9	3,33	+	4,44	+	4,11	+	23,3	+	81,0	+±4	210,0	+++
	1967	10	2,66	+	2,99	+	4,0	+	23,3	+	83,9	+±4	246,0	+++
Orthocid 50 Chemia-Viena (Captan)	1965	8	4,5	+	6,1	+	5,0	+	0	—	1,0	+	423,0	+++
	1966	9	3,40	+	4,5	+	4,0	+	0	—	0,6	+	399,0	+++
	1967	10	2,5	+	2,7	+	2,5	+	0	—	0,6	+	438,0	+++

Maximele de proiectare ale ascosporilor s-au înregistrat la înfoierea corolei, continuîndu-se în timpul înfloritului.

În zilele cu ploi peste 0,1 mm, s-au constatat proiectări masive de ascospori.

Între anii 1965—1967 s-au organizat experiențe de combatere folosind zeama bordeleză 0,5% și Captan (Orthocid 50 Chemia) 0,25%. La tratamentele cu zeamă bordeleză în concentrație de 0,5%, s-au constatat 2,66—3,33% frunze cu urme de atac (intensitate +) 1,99—5,96% fructe cu urme de atac și 4,0—5,11% lăstari foarte slab atacați (intensitate +). S-a înregistrat o producție medie pe variantă de 129—246 kg, fiind semnificativă față de cea realizată la pomii netratați. Pomii tratați cu zeamă bordeleză, au prezentat însă 23,3—25,6% frunze cu arsuri și 81,4—83,9% fructe cu suberificări care au cuprins de la 25% pînă la întreaga suprafață a fructului, depreciindu-le calitatea (tabelul 2).

La tratamentele cu Captan în concentrație de 0,25% s-a constatat 2,5—4,5 frunze cu urme de atac (intensitate +), 2,7—6,1% fructe cu urme de atac și 2,5—5% lăstari foarte slab atacați (tabelul 2). La acești pomi s-au recoltat 399—438 kg, fructe în medie pe variantă, producția fiind foarte semnificativă față de pomii de control netratați, la care s-a recoltat 32—54 kg fructe în medie pe variantă.

Captanul nu a produs arsuri pe frunze, iar fructele au avut 0,6—1,0% urme de suberificări (fig. 1 tabelul 2).

La pomii de control netratați împotriva rapănului, s-au înregistrat 35,5—51,7% frunze cu atac, 56—72% fructe puternic atacate și 52—62% lăstari atacați.

În anul 1968 s-au făcut experiențe cu 8 fungicide, comparîndu-le cu pomii de control netratați și pomii de control tratați cu zeamă bordeleză 0,5%. Cele mai bune rezultate în combaterea rapănului s-au obținut cu produsele pe bază de Captan (Orthocid 50 Chemia) la care s-au înregistrat 1,76—3% frunze cu urme de atac și 3,85—9,65% fructe cu urme de atac (tabelul 3).

La acest produs s-a observat 0,99 frunze cu urme de arsuri și 82% fructe fără suberificări.

Rezultate foarte bune s-au înregistrat și la produsul Melprex pe bază de dodecil guanidin, care a avut 0,44% frunze cu urme de atac, 7,63% fructe cu urme de atac (fig. 2) și 5% lăstari cu urme și la care s-au observat 0,45% frunze cu ușoare urme de arsuri și 92,37% fructe fără suberificări, producția medie pe variantă fiind de 514 kg fructe, fiind foarte semnificativă față de pomii de control netratați — care au avut pe variantă numai 300,4 kg fructe de cea mai slabă calitate.

Fig. 1 — Fructe din soiul Curé tratate cu Captan (Orthocid 50)

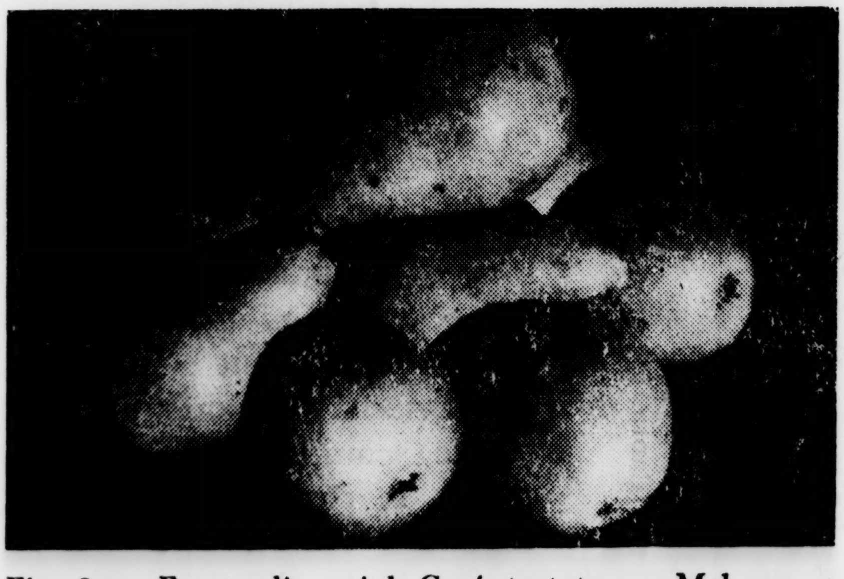


Fig. 2 — Fructe din soiul Curé tratate cu Melprex

Rezultate asemănătoare au fost obținute și la produsul pe bază de diclofluamidă — Euparen Bayer, la care au fost 0,57% frunze cu urme de atac, 8,88% fructe cu foarte slabe urme de atac și 5% lăstari foarte slab atacați. La acest produs au fost 0,94% frunze cu foarte slabe urme de arsuri și 90,4% fructe fără suberificări. Producția medie înregistrată a fost foarte semnificativă cu 666,5 kg fructe pe variantă.

La produsul pe bază de phaltan (Orthophaltan 50) s-au constatat 1,56% frunze cu urme de atac și 6,33% fructe cu slabe urme de atac, 0,3% frunze cu urme de arsuri și 91,2% fructe fără suberificări. S-au înregistrat 443,0 kg fructe în medie pe variantă, producția fiind semnificativă.

Tabelul 8

Eficacitatea câtorva fungicide folosite în combaterea rapănului la păr în anul *1968*, la staţiunea Voineşti

Varianta	Substanţa activă %	Concentraţia folosită la 100 l apă	Observaţii asupra atacului de rapăn pe:						Observaţii asupra fitotoxicităţii la:				Producţia	
			frunze		fructe		lăstari		frunze		fructe		media pe variantă kg	semnificaţia
			% atac	Int.	% atac	Int.	% atac	Int.	% atac	Int.	% atac	Int.		
Martor fără tratament	—	—	49,5	+ ±4	97,24	+ ±4	71,0	+ ±1	—	—	1,63	+	300,4	—
Zeamă bordeleză	SO_4 Cu şi var nestins	0,5 0,3	1,77	+	5,55	+	3,77	+	20,55	+	96,6	+ ±4	312,0	—
Orthocid 50 Chemia-Viena (Captan) (Austria)	N. triclormetil tiotetra hidroftalimidă	0,25	2,96	+	9,65	+	3,1	+	0,99	+	18,0	+	529,0	+++
Orthophaltan 50 (Schering-R.F.G.)	N. triclormetiltioftalimidă	0,2	1,76	+	6,33	+	5,0	+	0,3	+	8,6	+	443,0	+
Selene (Azzuro-Italia)	Etilen bis ditio carbamat de Zn	0,25	1,86	+	12,63	+	4,5	+	0,5	+	9,4	+	366	—
Aspor (Montecatini Italia)	Etilen bis ditiocarbamat de Zn	0,3	2,0	+	13,0	+	4,2	+	0,5	+	10,0	+	415,0	—
Euparen (Bayer-R.F. a Germaniei)	N.N. dimetil N. phenil-fenol diclor metilsulfamidă	0,1	0,57	+	8,88	+	5,0	+	0,94	+	9,6	+	666,5	+++
Cuman (CIBA-Elveţia)	Dimetil ditio carbamat de Zn.	0,2	2,05	+	18,0	+	4,0	+	0,85	+	9,9	+	386,0	—
Melprex	Acetat N. dodecil guanidină	0,1	0,44	+	7,63	+	5,0	+	0,45	+	7,63	+	514,0	+++

Rezultate bune față de martorul netratat, dar inferioare produselor anterioare, s-au obținut la produsele pe bază de zineb: Selene Azzuro-Valsele și Aspor-Montecatini.

Produsele pe bază de Ziram, au dat rezultate inferioare produselor pe bază de captan, dodecil guanidin, diclofluanid, phaltan și zineb.

Zeama bordeleză, deși a avut o foarte bună eficacitate în ce privește combaterea bolii (înregistrîndu-se numai 1,77% frunze cu urme de atac și 5,55% fructe cu urme de rapăn) a produs însă arsuri pe 20,53% din frunze și suberificări grave la 96,6% din fructe (fig. 3). Fructele de la această variantă au fost mai mici, au prezentat deformări ceea ce a determinat scăderea producției și o calitate inferioară (tabelul 3).

3 — Fructe din soiul Curé tratate cu zeamă bordeleză

Fig. 4 — Fructe din soiul Curé martor (netratat)

La pomii de control, fără tratament s-au constatat 49,5% frunze puternic atacate ; 97,24% fructe atacate (fig. 4) și 71% lăstari. Producția medie pe variantă a fost de 300,4 kg.

În timpul păstrării fructelor în depozit obișnuit, după o lună de la recoltare, cele mai mici pierderi din cauza moniliozei (*Monilinia fructigena* (Aderch. et Ruhl.) Honey), s-au constatat la fructele provenite din pomii tratați cu produsele : Orthocid 50, Euparen, Melprex și Zeamă bordeleză, care au avut pînă la 1,06% fructe putrezite. La fructele provenite din pomii de control netratați s-au înregistrat 19,43% fructe putrezite.

BIBLIOGRAFIE

1. *Aerts René, Soenen Albert* — Trois ans d'experimentation sur la tavelure du poirier. — *Venturia pirina* Ad. Agricultura Belgia, 1961, 9 no. 2.
2. *Berecki Z.* — Rozvoj stdium workowego i Konidialnego grzyba *Venturia pirina* Aderh. (Fusicladium pirinum Fuck). jako z ródla infekeji pierwotnej dla grusz. Parce instytutu sandownictva w Skierniewicach. Tom II 1957 — Warszawa.
3. *Cazarini Bruno* — Calendari dei trattamenti antiparassitari ai fruttiferi — Bologna, 1963.

4. *Darpoux H. et Perot A.* — Sur une méthode de traitements combinées curatifs préventife conte la tavelure du poirier. Phytiatrie — Phytopharmacie no. 4/1955, Versailles.
5. *Darpoux H. et Perot A.* — Essais comparatifs de traitements par pulvérisation ordinaire et par jet porté contre la tavelure du poirier. Phytiatrie — Phytopharmacie no. 4/1955.
6. *Darpoux A., Bulit J.* — Condition de réussits des traitements contre les maladies des arbres fruitiers a pépins. B.T.I.H.S. 1956, Station Centrale de Pathologie végetale I.N.R.A.
7. *Săvulescu A., Bontea V., Hulea A., Becerescu D., Marin A., Şuta V., Piersică E.* — Einfluss der Klimatischen Betingungen auf die Reifung der Perithezien von *Endostigme inaequalis* (Cooke) Syd. und auf das Auschleudern der Ascosporen. Phytopathologische Zeitschrift. Band 26. Heft 4/1956.
8. *Săvulescu Tr. şi colab.* — Starea fitosanitară în R.P.R. în anii 1928—1958, Bucureşti
9. *Székely I., Lefter Gh.* — Cercetări asupra biologiei şi combaterii ciupercii *Endostigme pirina* (Aderh.) Syd. Analele I.C.H.V., vol. III
10. *Şuta V., Moruju Gh., Morea I., Nica St., Rădulescu C., Mateescu N.* — Sensibilitatea cîtorva soiuri de măr, păr şi cireşi, la atacul principalelor boli în primii 9 ani de la plantare, la staţiunea Voineşti (1951—1959), Lucrări ştiinţifice I.C.C.A.-I.C.H.V., vol. IV, Bucureşti, 1965
11. *Da Tripoli Italo* — Considerazioni generalis sulla difesa fitosanitaria nell'annata 1963. Calendario dei tratamenti preflorali CIFF anno XIX nr. 1 Ferrara, 1964.
12. *Ubriszy G.* — Növénykórtan, Budapest, 1965

Bolile și dăunătorii care atacă Gerbera și combaterea lor

Ing. V. LEMENI
Stațiunea experimentală legumicolă Ișalnița—Craiova

Gerbera Jamesonii este o plantă floricolă foarte apreciată ca flori tăiate, cîștigînd multă popularitate în ultimii ani. Extinderea în cultură a acestei plante este mult stînjenită de atacul produs de boli și dăunători. Pentru a veni în ajutorul cultivatorilor și tuturor iubitorilor de gerbera prezentăm principalele boli și dăunători care atacă această plantă, precum și măsurile ce trebuie luate pentru a evita pagubele ce le produc.

Cercetările făcute în străinătate și în țara noastră au reușit să dea cîteva indicații pentru a se evita pagubele mari produse de agenții patogeni. La reușita unei culturi de gerbera trebuie să se aibe în vedere următoarele : să se cultive numai plante sănătoase, cele atacate să fie înlocuite ; să fie răspîndite și cultivate plantele (colonii) rezistente la boli ; înainte de plantare solul destinat cultivării acestei plante trebuie să fie dezinfectat ; să se aplice tratamentele l:

Dintre bolile și dăunătorii care a
frecvent întîlnite sînt :

FUZARIOZA, boală produsă de
tele de gerbera în toate stadiile de de:
zirea plantelor, care începe cu frunzele
Boala are un caracter mai grav cînd ata
se ofilesc, cad și în scurt timp se usucă
pe resturile de plante moarte. Plantele
truse rapid.

OFILIREA PLANTELOR este c
Verticilum albo-atrum, ea apare de ol
1—2 luni de la plantare. Se manifestă
mai accentuată ziua, iar noaptea își r
prin veștejirea frunzelor de la margine.
frunzei și astfel se împiedică circulația
pețiolul frunzei în dreptul vaselor con
galbenă brunie. În cazul atacului de ve
fuzarium la care pieirea este mult mai raț

LEMENI, V.

Bolile și dăunătorii rare ataca
lor. [Diseases and pests of
control.]

Revta Hortic. Vitic., 18 (4):82
Stat. exp. Legum. Isalnit

Microfilm no. 1562

O altă boală foarte periculoasă pentru această plantă este ofilirea provocată de Phytophthora cryptogea descoperită în anul 1937, în S.U.A., iar în anul 1959, a fost constatată și în R.D. Germană. Această boală produce distrugerea plantelor prin vestejirea și putrezirea rădăcinilor. Plantele atacate sînt distruse în scurt timp. În afară de bolile amintite mai sus, plantele de gerbera pot fi atacate și de putregaiul plantulelor (Pythium de Baryanum), căderea răsadului (Rhizoctonia violacea) făinarea și rugina.

Condițiile care favorizează apariția și evoluția bolilor în seră sînt : umiditatea excesivă, densitatea prea mare a plantelor, temperatura prea ridicată, aciditatea solului etc.

Pentru prevenirea şi combaterea acestor boli, se recomandă a fi luate următoarele măsuri :

— plantele atacate se vor înlătura şi distruge împreună cu pămîntul din jur ;

— din două în două săptămîni plantele se vor curăţa atent iar frunzele decolorate şi pătate se vor înlătura prin tăiere ;

— solul în care se cultivă gerbera trebuie să aibă un pH de 5—6,5 creîndu-se în acest fel plantelor o rezistenţă mai mare ;

— se recomandă înlocuirea pămîntului infectat, sau dezinfectarea lui cu 14—20 zile înainte de însămînţare sau plantare cu o soluţie de formalină în concentraţie de 2,5%, sau Captan în concentraţie de 0,6%, dîndu-se 10 litri/mp.

Dezinfectarea solului se mai poate face la o adîncime de 30—40 cm cu aburi, la temperatura de 90—95° C, timp de 20—30 minute. Prin aplicarea dezinfectării solului cu vapori de apă, s-au obţinut rezultate foarte bune, procentul de plante atacate a fost redus la 0,8—1,5%.

În timpul perioadei de vegetaţie se vor aplica tratamente la interval de 2 săptămîni cu Zineb 0,3%, Captan 0,2%, Lirocarmazin 0,2%.

Dintre dăunătorii cei mai întîlniţi în cultura de gerbera sînt :

Păianjenul roşu (Tetranychus urticae) foarte periculos atacînd un număr mare de plante. În ţara noastră produce pagube importante la o serie
în sere şi adăposturi de material plastic, printre
a atacului se produce o depigmentare (fig. 1)
duce în cele din urmă la o stagnare în creş-
ntelor.
combaterii acestui dăunător la staţiunea expe-
s-au executat stropiri repetate cu Telion V 18,
2% alternînd cu Bi 58 în concentraţie de

ate combate cu Phencapton 20 în concentraţie
ntraţie 0,1—0,2% şi Fac. 20 în concentra-

ă (Trialeurodes vaporariorum) este o insectă
en presărat şi cu o secreţie ceroasă de culoare
gale ca mărime.
această insectă poate produce pagube mari.
nţeapă şi sug seva din frunze care apoi îngăl-
ucă. Cînd atacul este mare întreaga plantă se
lezvoltare, iar recolta de flori este compromisă.
seră (Heliothrips haemorrhoidalis) este foarte
se manifestă prin pete alb-gălbui sau roşcate, care devin lucioase, datorită sugerii sucului celular. Frunzele atacate se necrozează, se brumifică parţial sau total şi în cele din urmă se usucă. Plantele atacate nu mai cresc şi rămîn mici.

1969
atacă berbera şi combaterea
ts of berbera and their
4):82-85. [4 fig.
alnita-Craiova.]
24

Păduchii de fruză se întîlnesc destul de des în cultura de gerbera. Atacul lor se manifestă pe frunzele şi bobocii florilor. Plantele atacate înfloresc slab, nu mai cresc şi chiar se îngălbenesc.

Buha legumelor (Plusia Gamma), dăunător care produce pagube însemnate culturilor de gerbera. Omida fluturelui gamma produce de asemenea pagube însemnate acestei culturi. A fost observat în cursul lunii

Bolile şi dăunătorii care atacă Gerbera şi combaterea lor

Ing. V. LEMENI
Staţiunea experimentală legumicolă Işalniţa—Craiova

Gerbera Jamesonii este o plantă floricolă foarte apreciată ca flori tăiate, cîştigînd multă popularitate în ultimii ani. Extinderea în cultură a acestei plante este mult stînjenită de atacul produs de boli şi dăunători. Pentru a veni în ajutorul cultivatorilor şi tuturor iubitorilor de gerbera prezentăm principalele boli şi dăunători care atacă această plantă, precum şi măsurile ce trebuie luate pentru a evita pagubele ce le produc.

Cercetările făcute în străinătate şi în ţara noastră au reuşit să dea cîteva indicaţii pentru a se evita pagubele mari produse de agenţii patogeni. La reuşita unei culturi de gerbera trebuie să se aibe în vedere următoarele : să se cultive numai plante sănătoase, cele atacate să fie înlocuite ; să fie răspîndite şi cultivate plantele (colonii) rezistente la boli ; înainte de plantare solul destinat cultivării acestei plante trebuie să fie dezinfectat ; să se aplice tratamentele la timp şi în bune condiţii.

Dintre bolile şi dăunătorii care atacă cultura de gerbera cele mai frecvent întîlnite sînt :

FUZARIOZA, boală produsă de *Fusarium oxysporum*, atacă plantele de gerbera în toate stadiile de dezvoltare. Se manifestă prin putrezirea plantelor, care începe cu frunzele şi pedunculii florali de la bază. Boala are un caracter mai grav cînd atacă plantele tinere. Plantele atacate se ofilesc, cad şi în scurt timp se usucă. Agentul patogen trăieşte saprofit pe resturile de plante moarte. Plantele atacate de această boală sînt distruse rapid.

OFILIREA PLANTELOR este o altă boală produsă de ciuperca Verticilum albo-atrum, ea apare de obicei în cultura de gerbera după 1—2 luni de la plantare. Se manifestă prin veştejirea plantelor care este mai accentuată ziua, iar noaptea îşi recapătă turgescenţa. Se manifestă prin veştejirea frunzelor de la margine. Ciuperca astupă vasele din ţesutul frunzei şi astfel se împiedică circulaţia sevei. Dacă se face o secţiune prin peţiolul frunzei în dreptul vaselor conducătoare acestea prezintă o zonă galbenă brunie. În cazul atacului de verticilium plantele pier lent faţă de fuzarium la care pieirea este mult mai rapidă.

O altă boală foarte periculoasă pentru această plantă este ofilirea provocată de Phytophthora cryptogea descoperită în anul 1937, în S.U.A., iar în anul 1959, a fost constatată şi în R.D. Germană. Această boală produce distrugerea plantelor prin vestejirea şi putrezirea rădăcinilor. Plantele atacate sînt distruse în scurt timp. În afară de bolile amintite mai sus, plantele de gerbera pot fi atacate şi de putregaiul plantulelor (Pythium de Baryanum), căderea răsadului (Rhizoctonia violacea) făinarea şi rugina.

Condiţiile care favorizează apariţia şi evoluţia bolilor în seră sînt : umiditatea excesivă, densitatea prea mare a plantelor, temperatura prea ridicată, aciditatea solului etc.

Pentru prevenirea şi combaterea acestor boli, se recomandă a fi luate următoarele măsuri :

— plantele atacate se vor înlătura şi distruge împreună cu pămîntul din jur ;

— din două în două săptămîni plantele se vor curăţa atent iar frunzele decolorate şi pătate se vor înlătura prin tăiere ;

— solul în care se cultivă gerbera trebuie să aibă un pH de 5—6,5 creîndu-se în acest fel plantelor o rezistenţă mai mare ;

— se recomandă înlocuirea pămîntului infectat, sau dezinfectarea lui cu 14—20 zile înainte de însămînţare sau plantare cu o soluţie de formalină în concentraţie de 2,5%, sau Captan în concentraţie de 0,6%, dîndu-se 10 litri/mp.

Dezinfectarea solului se mai poate face la o adîncime de 30—40 cm cu aburi, la temperatura de 90—95° C, timp de 20—30 minute. Prin aplicarea dezinfectării solului cu vapori de apă, s-au obţinut rezultate foarte bune, procentul de plante atacate a fost redus la 0,8—1,5%.

În timpul perioadei de vegetaţie se vor aplica tratamente la interval de 2 săptămîni cu Zineb 0,3%, Captan 0,2%, Lirocarmazin 0,2%.

Dintre dăunătorii cei mai întîlniţi în cultura de gerbera sînt :

Păianjenul roşu (Tetranychus urticae) foarte periculos atacînd un număr mare de plante. În ţara noastră produce pagube importante la o serie de plante floricole cultivate în sere şi adăposturi de material plastic, printre care şi gerbera. Ca urmare a atacului se produce o depigmentare (fig. 1) puternică a frunzişului care duce în cele din urmă la o stagnare în creştere, la uscarea şi pieirea plantelor.

Pentru buna reuşită a combaterii acestui dăunător la staţiunea experimentală legumicolă Işalniţa s-au executat stropiri repetate cu Telion V 18, în concentraţie de 0,15—0,2% alternînd cu Bi 58 în concentraţie de 0,075—0,1%.

Păianjenul roşu se poate combate cu Phencapton 20 în concentraţie de 0,1%, Kheltan în concentraţie 0,1—0,2% şi Fac. 20 în concentraţie de 0,2%.

Musculiţa albă de seră (Trialeurodes vaporariorum) este o insectă mică 2 mm) cu corpul galben presărat şi cu o secreţie ceroasă de culoare albă. Adulţii au patru aripi egale ca mărime.

În cultura de gerbera această insectă poate produce pagube mari. Atît insectele cît şi larvele înţeapă şi sug seva din frunze care apoi îngălbenesc, se răsucesc şi se usucă. Cînd atacul este mare întreaga plantă se îngălbeneşte stagnează din dezvoltare, iar recolta de flori este compromisă.

Tripsul plantelor de seră (Heliothrips haemorrhoidalis) este foarte răspîndit în seră. Atacul se manifestă prin pete alb-gălbui sau roşcate, care devin lucioase, datorită sugerii sucului celular. Frunzele atacate se necrozează, se brumifică parţial sau total şi în cele din urmă se usucă. Plantele atacate nu mai cresc şi rămîn mici.

Păduchii de fruză se întîlnesc destul de des în cultura de gerbera. Atacul lor se manifestă pe frunzele şi bobocii florilor. Plantele atacate înfloresc slab, nu mai cresc şi chiar se îngălbenesc.

Buha legumelor (Plusia Gamma), dăunător care produce pagube însemnate culturilor de gerbera. Omida fluturelui gamma produce de asemenea pagube însemnate acestei culturi. A fost observat în cursul lunii

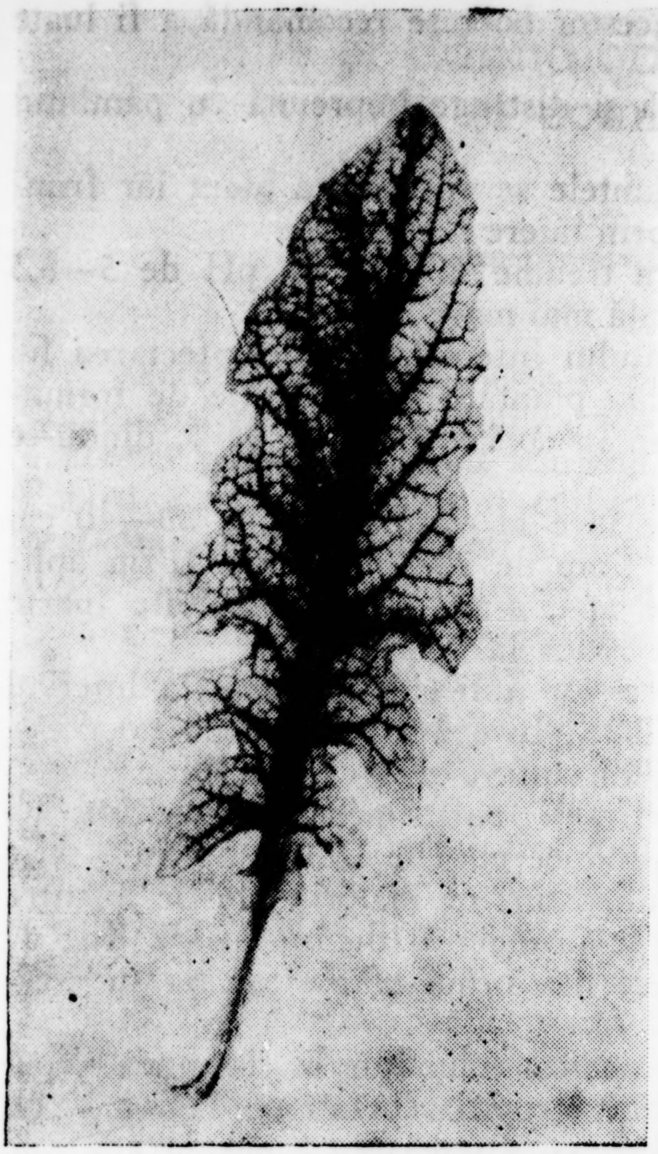


Fig. 1 — Atac de Tetranychus urticae pe frunzele de Gerbera jamensonii

Fig. 2 — Atac de Plusia gama la frunze

iunie—august, se manifestă prin rosături asupra frunzelor (fig. 2). Cel mai periculos atac este atunci cînd larvele atacă floarea (fig. 3), consumînd petalele florilor.

Ca măsuri de combatere împotrica acestor dăunători se vor explica la apariţia lor stropiri repetate cu Ekatox 50 în concentraţie de 0,06—0,08%, Lirothion 50 concentraţie de 0,06—0,08%, Bi 58 în concentraţie de 0,075—0,1%, Carbatox în concentraţie de 0,1—0,3%.

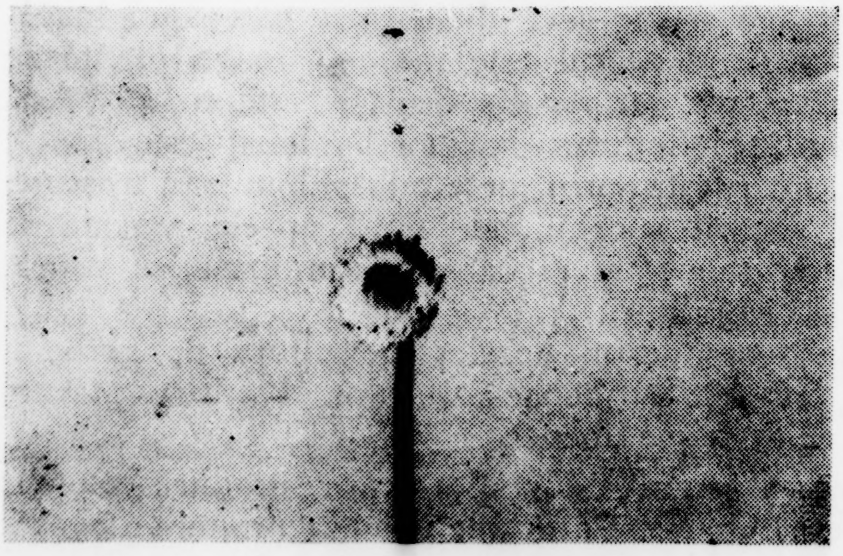


Fig. 3 — Atac de Plusia gama la floare

Musculiţa mineră (Liromyza sp.) este un dăunător puţin cunoscut în ţara noastră dar care a început să producă pagube destul de mari. Pentru prima dată a fost observat în culturile de gerbera de la staţiunea experimentală legumicolă Işalniţa.

Insecta adultă asemănătoare cu nişte musculiţe mici, cu lungimea corpului de 2—2,5 mm, avînd larvele mici de culoare galben-verzuie.

Plantele de gerbera atacate, au frunzele gălbui prezentînd urme întortochiate mai deschise la culoare şi cu dungi de excremente negre (fig. 4). Plantele atacate sînt mult stînjenite din dezvoltare şi dau flori de slabă calitate.

Fig. 4 — Atac de musculiţă mineră (Liromyza sp.)

Combaterea acestui dăunător s-a făcut prin stropiri cu Bi 58 în concentraţie de 0,1% şi cu Lirthion 50 în concentraţie de 0,08%.

Tratamentele aplicate contra bolilor şi dăunătorilor din cultura de gerbera se pot aplica combinat, insecticide cu fungicide, realizîndu-se economii de soluţie şi este uşor de aplicat dar trebuie avut în vedere compatibilitatea substanţelor. Prin cunoaşterea acestor boli şi dăunători, cultivatorii de gerbera vor putea interveni la timp, luînd măsurile corespunzătoare în scopul obţinerii unor producţii de flori de calitate superioară contribuind în acest fel la mărirea veniturilor.

LONZA

Obstbau

C

Flüssig
Mit Netzmittel
Hochwirksam

LONZA

Inhalt

Seite

Methoden zur Verhütung von Jonathan-Spot
K. Stoll 365

Die Bewertung von Obstanlagen und Obstbäumen
A. Wirth, T. Meli und H. P. Späti 371

Separatoren in der Kellerwirtschaft, ihre Konstruktion und Funktion
F. Dellsperger 377

382

384

387

392

Eine interessante Vorführung von Rebbaumaschinen und -geräten im Rebberg der landw. Schule Wülflingen 392

Rotbrenner-Spätinfektionen bei Reben 395

Mitteilungen und Veranstaltungen

Degustationsstand für Ostschweizer Weine an der OLMA 1969 396

Statistik 397

STOLL, K. 1969

Methoden zur Verhütung von Jonathan-Spot. [Methods for the prevention of Jonathan spot.]

Schweiz. Z. Obst- u. Weinb., 105 (16):365-371. [2 fig., 4 tables. Eidg. Forschungsanstalt, Wädenswil.]

Microfilm no. 15625

Schweizerische Zeitschrift für Obst- und Weinbau

einschliesslich Verwertung

Nummer 16   26. Juli 1969   105. Jahrgang

Organ des Schweiz. Obstverbandes und des Schweiz. Weinbauvereins

Erscheint jeden zweiten Samstag. Einmal monatlich mit Beilage Obstrundschau

Redaktion: Direktor Dr. R. Fritzsche, E. Peyer, Dr. A. Wirth und H. Tanner, Eidg. Forschungsanstalt für Obst-, Wein- und Gartenbau in Wädenswil, Tel. 051 / 75 13 33

Der Nachdruck von Artikeln, auch nur auszugsweise ist nur bei vollständiger Quellenangabe gestattet.

Methoden zur Verhütung von Jonathan-Spot

Von K. Stoll, Eidg. Forschungsanstalt, Wädenswil

Jenes alterungsbedingte Krankheitssyndrom der Apfelhaut, welches unter dem Sammelnamen Jonathan-Spot bekannt ist, manifestiert sich in zwei Hauptausprägungsformen: den kurz nach der Ernte auftretenden **Baumspot** und den nach einigen Monaten Lagerdauer sich zeigenden **Lagerspot.** Vor allem das

Abb. 1 **Baumspot** Kreisrunde, kleine, nur 1 bis 4 mm Durchmesser zeigende Flecken an Lentizellen und Hautrissen. Fast schwarz und etwas in die Haut vertieft. Vor allem an der besonnt gewesenen Fruchthälfte.
Tritt bei zu spätem Pflücken schon am Baum auf, vor allem aber in den ersten Lagermonaten. Ist oft Folge eines zu späten Pflückens. (Foto R. Isler)

Abb. 2 **Lagerspot** Grössere Flecken, Ringe und Streifen in mattbrauner Farbe. Gar nicht oder nur sehr wenig eingesunken in die Haut, aber an der ganzen Fruchtoberfläche vorkommend.
Tritt erst nach Mitte der üblichen Lagerzeit auf. Ist das Kennzeichen der zu lang dauernden Lagerung der Frucht.
(Foto R. Isler)

Krankheitsbild und die zu praktizierenden Verhütungsmassnahmen sind es, welche eine solche Unterteilung als sinnvoll erscheinen lassen.

Die tieferen Ursachen des Auftretens von Jonathan-Spot sind nicht restlos erforscht. Viele Anzeichen deuten darauf hin, dass es sich um Aufbrauchs- und Ueberalterungsschäden handelt. Beim Baumspot können belastende Aussenfaktoren, wie beispielsweise stark wechselnde Wasserversorgung, lokale Austrocknung durch grelle Sonneneinwirkung usw., mitspielen. Der Feinbau der Haut in der Zone der Lentizellen kann selbst durch die Wahl verschiedener Spritzmittel beeinflusst sein. So bestand in einem vergleichenden Fungizidversuch eine auffallende Parallelität des Auftretens von Lentizellenröte an 'Golden Delicious' und von Baum-Spot an 'Jonathan' (1).

Der Lentizellenröte liegen nach eigenen Beobachtungen und einer eingehenden Untersuchung (2) primär die Hautrisse zugrunde. Es wäre näher zu prüfen, ob der Baumspot nicht eine Parallelerscheinung darstellt. Mit heranrückender Baumreife hat die Apfelhaut schon viel von ihrer Elastizität und Anpassungsfähigkeit an wechselnde Belastungen verloren. Allfällig auftretende Hautrisse oder lokale Austrocknungen sind durch die Frucht selbst nicht mehr vollständig reparierbar. Verursacht wird der Baumspot also wahrscheinlich durch gewisse strukturelle Mängel im Lentizellengewebe, welches dadurch vorzeitig altert und sich schwarz verfärbt.

Der Lagerspot dagegen ist als echtes Ueberalterungssymptom anzusprechen. Ein zu spätes Pflücken der Frucht, der ausgedehnte Lagerungsverzug zwischen Ernte und Kühlung, hohe Lagertemperaturen und lange Lagerzeiten zehren an den Haltbarkeitsreserven der Frucht, was unweigerlich zur Erkrankung jener Hautpartien führt, welche den grössten Stoffwechselbelastungen ausgesetzt sind. Auch die Akkumulation der von der Frucht ausgeschiedenen Stoffwechselgase scheint negative Auswirkungen zu haben (Tabellen 1 und 2). Beim Testen von Lagerbehältern trat regelmässig in jenen Fällen starker Lagerspot auf, wo die natürliche Durchlüftung des Lagergutes eingeschränkt war.

Tabelle 1 Lager-Spot an 'Jonathan' und Hautbräune an 'Golden Delicious' in Lagerbehältern mit unterschiedlicher Durchlüftungsrate

(3 °C, 93 % rel. F., für 6 Monate plus 1 Woche 15 °C)

$$\text{Lagerspot- und Hautbräune-Index} = \frac{\%\ \text{schwach}}{4} + \frac{\%\ \text{mittel}}{2} + \frac{\%\ \text{stark}}{1}$$

Lagerbehälter	Saison 1966/67		Saison 1967/68		
	% ohne Lager-spot	Lagerspot-Index 'Jonathan'	% ohne Lager-spot	Lagerspot-Index 'Jonathan'	Hautbräune-Index 'Golden Delicious'
A. Holzkisten 11 kg, ohne Papiere	48	71	31	102	14
B. Holzkisten 11 kg, gelochte Bodenpapiere	45	76			
C. Holzkisten 11 kg, gelochte Boden- und Seitenpapiere	33	92			
D. Nestpack 9 kg aus Kunststoff	23	113	16	158	28
E. Polystyrolharasse 4 kg (1,5 % Löcher) *	21	117	19	125	38
F. Polystyrolharasse 4 kg (9 % Löcher) *			27	92	20

* Summe der freien Oeffnung in % der Aussenoberfläche der Gefässe.

Aus diesen Zahlen ist ersichtlich, dass in zu wenig durchlüftbaren Lagerbehältern (C, D, E) der Befall an Lagerspot absolut — und an Intensität — zunahm. Dies geschah in ähnlicher Weise wie beim Auftreten der Hautbräune an 'Golden Delicious' und 'Boskoop'.

Bei der Sorte 'Jonathan' ist die wirtschaftlich sinnvolle Lagerdauer speziell infolge der Spot-Krankheiten eine sehr begrenzte. Im Naturlager zeigen sich oft schon nach 2, im Kühllager nach 4 Monaten die Flecken in sehr grossem Ausmass. Auch die direkten Abkömmlinge von 'Jonathan', wie 'Idared' und 'King David', sind nicht immer immun gegen diese Störung. Hier treten aber erst dann Verlustprobleme auf, falls diese Sorten eindeutig zu spät vermarktet werden.

Mögliche Verhütungsmassnahmen

1. Empfehlungen genereller Natur

Unter allen Umständen hat sich als nützlich erwiesen, die Früchte im Stadium der knappen Pflückreife zu ernten und diese sofort einzukühlen auf die passendste Lagertemperatur. Für schweizerische Anbauverhältnisse müssen wir + 3 bis 4 ° empfehlen. Früchte aus wärmeren Gebieten — wie Italien und Ungarn — ertragen auch eine Lagertemperatur zwischen 2 bis 3 °C.

Der Entfernung der Stoffwechselgase aus dem Lagerraum mittels genügender Frischluftzufuhr oder der Luftwäsche mit Wasser ist die gebührende Aufmerksamkeit zu schenken (3).

Tabelle 2 Reduktion der Jonathan-Spot-Indices in Kühllagern mit Luftwäsche (3 °C, 93 % rel. F., 4—6 Monate)

Kühllager	1958	1959	1960 *	1961	1962	1965
ohne Luftwäsche	22	6	69	45	29	49
mit Luftwäsche	10	3	89	35	7	30

* 1960 gepflückt in einem zu reifen Stadium.

Mit Lufterneuerung oder der Luftwäsche allein wird das Auftreten von Jonathan-Spot wohl gemildert, der Schaden ist aber nicht völlig verhütbar. Falls nicht die nacherwähnten speziellen Verhütungsmethoden in Frage kommen, hat man die Lagerposten häufig zu kontrollieren. Das erste Auftreten von Spot muss als Startzeichen gelten für die Vermarktung der Ware.

2. Lecithin-Tauchbäder vor der Einlagerung

Angeregt durch Arbeiten des holländischen Fachmannes vom Sprenger-Institut in Wageningen, Dr. O. L. STADEN (4), haben wir auch **Lecithin-Pimaricin-Tauchbäder** getestet. Dem Lecithin-Präparat der holländischen Lieferfirma ist das Fungizid Pimaricin beigefügt zur Schimmelverhütung auf dem Tauchbelag.

Wie die Zahlen der Tabelle 3 zeigen, war der Erfolg offensichtlich: Die unbehandelten Kontrollmuster zeigten nur 33 bis 43 % völlig fleckenfreie Aepfel,

Tabelle 3 Wirkung von Lecithin-Pimaricin-Tauchbädern gegen Lagerspot

Behandlungsart	% ohne Spot	% ohne Spot + leichter Spot	% starker Spot	Spot-Index
Saison 1966/67				
177 Lagertage bei 4 °C, plus 7 Tage bei 15 °C				
Unbehandelt	43	68	14	37
Lecithin-Pimaricin 0,5 %	82	90	0	8
Lecithin-Pimaricin 2,5 %	82	91	2	8
Saison 1967/68 A				
188 Lagertage bei 4 °C, plus 13 Tage bei 15 °C				
Unbehandelt	33	54	20	82
Lecithin-Pimaricin 1 %	64	77	2	30
Lecithin-Pimaricin 1,5 %	70	89	0	19
Lecithin-Pimaricin 2 %	77	91	0	15
Lecithin-Pimaricin 2,5 %	87	100	0	4
Saison 1967/68 B				
179 Lagertage bei 4 °C, plus 13 Tage bei 15 °C				
Unbehandelt	42	68	11	55
Lecithin-Pimaricin 0,5 %	76	90	1	17
Lecithin-Pimaricin 1 %	78	92	0	13
Lecithin-Pimaricin 1,5 %	77	93	0	14
Lecithin-Pimaricin 2 %	84	95	0	9
Lecithin-Pimaricin 2,5 %	85	96	0	9

die mit optimaler Konzentration behandelten zwischen 82 und 87 %. Gesetzt den Fall, dass das Vorhandensein von 1 bis 3 kleinen unscheinbaren Flecken vom Markt toleriert wird, könnten die behandelten Muster zu über 90 % marktfähiger Früchte darstellen. Mit starken Flecken behaftete Früchte waren nur noch vereinzelt oder überhaupt nicht vorhanden. Wie mit steigender Konzentration der Tauchlösung die Wirksamkeit verbessert wurde, ist aus der Kolonne der Spot-Indices ersichtlich.

Die Wirksamkeit des Lecithins kann entweder auf einer direkten Einwirkung ins Stoffwechselgeschehen der Frucht beruhen oder aber — was uns als wahrscheinlicher erscheint — mittels Bildung eines gaswechselhemmenden Filmes auf der Fruchtoberfläche die Alterung der Frucht auf indirekte Weise verzögern, indem es zu einer CO_2-Anreicherung/resp. O_2-Verarmung innerhalb des Filmes kommt. Lecithin-Tauchbäder sind nämlich auch wirksam zur Reduktion der Hautbräune an jenen Apfelsorten ('Boskoop', 'Golden Delicious'), welche den «Ueberalterungstyp» der Hautbräune zeigen und welcher weitgehend verhütbar ist durch Lagerung der Früchte in kontrollierter Atmosphäre. Der «Unreifetyp» der Hautbräune ('Roter Delicious', 'Stayman', 'Bohnapfel') ist dagegen nicht verhütbar mit Lecithin, und dieser Typ wird in kontrollierter Atmosphäre eindeutig akzentuiert.

3. Tauchbäder anderer Art

Das Eintauchen der Früchte vor der Lagerung in ein Heisswasserbad (53 °C für 1 Minute oder 54 °C für 1/2 Minute) hat in mehreren Fällen zu 80 % fleckenfreier Ware geführt. In anderen Fällen waren entsprechende Heisswasserbäder völlig unwirksam. Diese Methode ist zuwenig zuverlässig.

Tauchbäder in Sonnenblumenöl (emulgiert mit Na-Laurylsulfat) ergaben rund 90 % fleckenfreie Ware. Weil ölig anzufühlende Früchte auf dem Markt von der Hausfrau abgelehnt werden, kann dieses Rezept kaum je in Frage kommen.

4. Die Lagerung in kontrollierter Atmosphäre (CA)

Wie die jahrelange kommerzielle Apfellagerung in gasdichten Kühlräumen beweist, kann diese Aufbewahrungsmethode den Jonathan-Spot in seinen beiden Ausprägungsformen total verhüten. Voraussetzung hiefür sind das rechtzeitige Ernten, das sofortige Einkühlen und das Einregulieren von **mindestens 3 % CO_2**. Die gleichzeitige Sauerstoffsenkung auf 3 bis 4 % ist zur Verhütung des Spots nicht notwendig. Dieselbe wirkt aber an sich zusätzlich reifehemmend, und das Scrubbersystem der CA-Lagerung wirkt sich allgemein vorteilhaft aus für die Geschmacksqualität der Früchte.

Das da und dort propagierte völlige Wegscrubbern des CO_2 in gasdichten Räumen ist für die Sorte 'Jonathan' nicht empfehlenswert.

Die Sauerstoffsenkung von 21 % auf 2 % vermochte das Auftreten von Jonathan-Spot wohl zu verzögern, aber nicht zu verhüten, denn wir brauchen hiefür bestimmte minimale Mengen an CO_2.

Da die Einrichtung gasdichter Kühlräume nicht jedem Lagerhalter zumutbar ist, begegnen gewisse Abwandlungen des CA-Systems einem gewissen Interesse. So haben wir im Winter 1968/69 'Jonathan'-Aepfel in Palettsäcken gemäss französischem Patent C.N.R.S. **Marcellin-Leteinturier** gelagert (5). Diese Kunststoffzelte fassen bis zu 1000 kg und sind mit einem **gasregulierenden Fenster aus Siliconrubber** versehen.*

Im 500-kg-Sack, der in einem Naturkeller aufgestellt war, mit anfänglich 14 °C, dann bis zum 15. 11. absinkend auf 7 °C und später auf 2 °C, konnten

Tabelle 4 Jonathan-Spot unter dem Einfluss variabler CO_2-Konzentrationen in der Lageratmosphäre

Jahr	Lagerungsart	Lagertage	% Spot an 'Jonathan'
1966/67	Kistenfutter PE 25 kg, bei 4 °C 4,7 % CO_2 / 15,8 % O_2 * (bei Auslagerung)	253	0
1966/67	CA-Obstlagerraum, 45 t bei 4 °C 4 % CO_2 / 4 % O_2	150	~ 0
1967/68	Kistenfutter PE 25 kg, bei 4 °C 3,8 % CO_2 / 17,0 % O_2 * (bei Auslagerung)	234	21
1967/68	Kistenfutter PE 25 kg, bei 2 bis 12 °C, Naturlager, 4,1 % CO_2 / 6,2 % O_2	150	0
1967/68	CA-Obstlagerraum 46 t, bei 4 °C 4 % CO_2 / 4 % O_2	190	~ 0
1968/69	Versuchsboxe 100 kg, bei 4 °C 3 % CO_2 / 3 % O_2	220	0
1968/69	Versuchsboxe 100 kg, bei 4 °C 0 % CO_2 / 2 % O_2	220	33
1968/69	Palettsack C.N.R.S. 970 kg, bei 3 °C 3—5 % CO_2 / 2—5 % O_2	189	~ 0
1968/69	Kontrolle bei 3 °C	189	51
1968/69	Palettsack C.N.R.S. 500 kg bei 2—11 °C 3—5 % CO_2 / 6—1 % O_2	176	~ 0
	Kontrolle bei 2—11 °C	176	59
	CA-Obstlagerraum 47 t, bei 4 °C 4 % CO_2 / 3,5 % O_2	180	~ 0

* Grosstechnisch entwickelt und kommerziell ausgewertet wird das Patent dieser Palettsäcke von der Firma Rhône-Poulenc. Schweizer Adresse: Rhodia S.A., 1211 Genève.

'Jonathan'-Aepfel praktisch spotfrei gelagert werden vom 14. Oktober bis zum 2. April. Der danebenstehende Kontrollposten wies 59 % spotbehaftete Früchte auf zum gleichen Zeitpunkt.

Im Kühllager +3 °C wurden 970 kg 'Jonathan' in einem Palettsack aufbewahrt, wobei sich hier die Atmosphäre auf 3 bis 4,5 % CO_2 und 2 bis 5 % O_2 einregeln liess. Auch hier trat praktisch kein Jonathan-Spot in Erscheinung.

Die extrem hohe relative Feuchtigkeit im Innern der Säcke hat im Kühllager zwar etwas CO_2-Aetzungshautbräune an den Früchten verursacht. Einzelne beim Einlagern stark reife Aepfel sind auch weich und morsch geworden. Die Auslagerungsergebnisse rechtfertigen aber eine Weiterprüfung dieses Lagersystems. Laut Angaben der Lieferfirma ist eine Nachkontrolle der CO_2/O_2-Gehalte in den Säcken empfohlen. Eigene Beobachtungen lassen diese periodischen Messungen als dringend notwendig erscheinen. Hiezu taugt das einfach zu bedienende CO_2/O_2-Fyrite-Messgerät (Firma C. Couteau, 15, Rue des Eaux-Vives, 1211 Genève).

Auch das Lagern der Jonathan-Aepfel in Polyäthylen-Kistenfutter zu 25 kg, welche bei 4 °C mit 3 cm Halsöffnung versehen werden, kann den Jonathan-Spot

vielfach verhüten, falls rechtzeitig eine CO_2-Anreicherung von 4 bis 6 % stattfindet. Da hier nur eine sehr knapp reife Ware (Gefahr des Morschwerdens!) berücksichtigt werden kann, wird sich diese Lagerungsmethode kaum durchsetzen.

Zusammenfassung

1. Jene bekannten herkömmlichen Empfehlungen zur Reduktion des Jonathan-Spots, wie das Ernten bei knapper Pflückreife, sofortiges Einkühlen auf die tiefstmögliche sichere Temperatur — wie auch gute Luftreinhaltung —, gelten unverändert weiter. Doch nur bei kurzfristiger Lagerung können diese Vorkehren genügen.
2. Eine Behandlung der Früchte vor der Lagerung mit Tauchlösungen aus Lecithin-Pimaricin vermochte den Jonathan-Spot mit guter Wirkung einzudämmen.
3. Die Lagerung der Jonathan-Aepfel in kontrollierter Atmosphäre bei mindestens 3 % CO_2 ist eine sichere Verhütungsmethode. Erfolgversprechend sind in dieser Hinsicht auch die Palettsäcke mit Lüftungsfenster nach dem System Marcellin-Leteinturier.

Literaturhinweise:

1 STOLL, K.: Schweiz. Zeitschr. Obst- und Weinbau **67**, S. 38, 1958.

2 BOHNEN, K.: Zeitschr. Pflanzenkrankheiten und Pflanzenschutz **74**, 602—615, 1967.

3 STOLL, K. und SCHÜEPP, H.: Proc. XI Int. Congr. Refrig. München, 1963. S. 1147—1150.

4 STADEN, O. L.: Annual Report I.B.V.T. Wageningen, 1959, S. 23.

5 MARCELLIN, P., LEBLOND, C., et LETEINTURIER, J.: Proc. 96, Congr. Pomologie de France, 1965, S. 91—114.

Die Bewertung von Obstanlagen und Obstbäumen

Von A. Wirth und T. Meli, Eidg. Forschungsanstalt, Wädenswil
und H. P. Späti, Schätzungsamt des Schweiz. Bauernverbandes, Brugg

(Fortsetzung und Schluss)

IV. Die Bewertung von Bäumen des Altobstbaues (Hochstämme)

1. Schätzung von Jungbäumen

Auch hier ist bis zum Eintritt ins eigentliche Ertragsalter der **Produktionskostenwert** massgebend. In Tabelle 4 sind die Produktionskostenwerte für junge Hochstammbäume zusammengestellt. Die Zahlen beziehen sich ebenfalls auf normal entwickelte Bäume. Wenn sie durch schlechte Baumpflege, schlechten Standort und Wunden usw. stark zurückgeblieben sind, so müssen die Standjahre entsprechend reduziert werden. Die ziemlich weite Spanne innerhalb der aufgeführten Von-bis-Werte lassen im übrigen der Berücksichtigung der effektiven Verhältnisse bezüglich Standort und Pflegezustand genügend Raum. Es sei hier speziell daran erinnert, dass der Produktionskostenwert eine Aussage über die bisherigen Aufwendungen, weniger aber Angaben über künftige Produktionsmöglichkeiten gibt. So ist z. B. der effektive Wert eines Jungbaumes im Streuobstbau für denjenigen, der ihn übernehmen muss, praktisch gleich null.

MATERIAL UND ORGANISMEN MATERIALS AND ORGANISMS · MATÉRIAUX ET ORGANISMES

Herausgeber: Prof. Dr. Günther Becker · Redaktionssekretär: Dr. Helmut Kühne
Bundesanstalt für Materialprüfung, 1 Berlin 45 (Dahlem), Unter den Eichen 87

Verlag: Duncker & Humblot, 1 Berlin 41 (Steglitz), Dietrich-Schäfer-Weg 9, Postfach 330, Ruf: 72 04 26 — Die Zeitschrift erscheint viermal jährlich. Jedes Heft hat einen Umfang von 80 Seiten.

Alle Rechte, auch die des auszugsweisen Nachdrucks, der photomechanischen Wieder-
gabe und der Übersetzung, für sämtliche B
Buchdruckerei Union GmbH, 1 Be

SELL, J. & WÄLCHI, O. 1969

Changes in the surface texture of weather exposed wood.

- Mater. & Org., 4 (2): 81-87.

[2 fig., EMPA, Dübendorf, Switzerland.]

Microfilm No. 15626

M. Lenz: Zur schädigenden Wirkung einig

M. Fougerousse, J. Guillemain, C. Jacquiot,
Serment: Contribution à l'étude de l'effi
traités par trempage long dans des solu

F. A. Rosenberg and H. Breiter: The Role
Digestive Processes of the Shipworm (Pa

Besprechungen ....

Changes in the Surface Texture of Weather-exposed Wood*

By Jürgen Sell and Oskar Wälchli

EMPA Dübendorf and EMPA St. Gallen (Switzerland)

Contents

1. Technical effects
2. Aesthetical consequences
3. Summary
   Literature

When wood is exposed to solar radiation and liquid water, it undergoes, in a relatively short time, external changes which are macroscopically visible. This report describes the nature and origin of some principal weather effects and their secondary consequences.

1. Technical effects

Wood in open air constructions is usually unevenly exposed to changes of climate. The surfaces that are principally or occasionally exposed to the influences of the weather are considerably more heated by solar radiation (e. g. up to temperatures of 80° C), and accordingly more intensively wetted by precipitations, than the parts that are protected or less exposed to these influences. Owing to the one-sided heating of the wood there occur steep gradients of moisture content from the warmer to the colder side, as they were determined experimentally by R. Keylwerth (1966). The gradients of moisture across the section produce inconsistent tensions of shrinking and swelling which are absorbed by the deformations or the splitting of the wood, usually rather by both.

One-sided and unequal moistening of wood by water in liquid state also produces inconsistent distribution of tension, since the moisture content in the interior of the wood and in its averted zones is below that of the external ones. Formal and dimensional changes are the

* This report was written on behalf of the OECD-group of experts on Wood Preservation, Research Programme II — Fundamentals of Test Methods, which had decided to publish this report during the meeting held on Octobre 9th, 1968 in Paris.

consequences which may injure the constancy of the dimensions of a wooden construction. In general the rate of deformation increases with the reduction of the section of the wood. In addition it depends to a great extent on some other parameters, among them the elastic and plastic behaviour of the wood, the mechanical resistance, the gradient of the moisture content, the structure of its cross section, the shrinking, the swelling, the sorptive behaviour of the type of wood and the duration of a climatic strain.

The development of microscopical cracks in wood of *Sequoia sempervirens* and *Pseudotsuga menziesii* by ultraviolet radiation and weathering has been investigated by V. P. Miniutti (1967). He found that degrading of wood is in particular caused by short wave ultraviolet rays, a process which affects spring wood tracheides more than summer wood tracheides. The cells with pits proved to be the most susceptible to photolysis, as the first cracks can be observed in their neighbourhood. Moreover, such cracks occur more often in wood of a high lignin content. As it is already known from earlier investigations (e. g. W. Sandermann and F. Schlumbom, 1962) the photolysis of lignin is more intensive than that of cellulose. V. P. Miniutti (1967) believes that microscopic cracks in UV-irradiated wood are due to a loss of wood substance caused by photolysis combined with shrinkage across the fibre and a loss of tension between tracheides. According to our own observations the above mentioned moisture gradients in wood that is one-sidedly exposed to the weather and the tensions resulting from this also play an important role. Above all the majority of the deeper cracks in the wood must have been produced by these tensions.

The unequal swelling (and erosion) of spring and summer wood of conifers is another effect due to the influence of weather. In the course of the exposure to the weather, the more strongly swelling summer wood undergoes stronger permanent deformation. In addition, owing to the comparatively small shear strength between the cells of the summer wood and these of the spring wood and to the more intensive erosion of the spring wood, there occurs the so-called "rising of the year-rings". The surface of the cross section assumes a serrated appearance.

As the dimensional and formal changes injure the constancy of structural wooden parts, cracks must be considered as places of infection for wood discolouring or wood destroying fungi, since the cracks often penetrate chemically protected external zones deep down into the interior of the wood. Occasionally large cracks may retain water. Film-forming coatings are much strained by a moving wooden base, in particular at the edges of cracks and at other uneven spots of the wooden surface.

2. Aesthetical consequences

Beside the above mentioned changes of the superficial structure of the wood, the change of colour is particularly striking, and mostly an aesthetically non-desired consequence of exposure to the weather. Every kind of wood exposed to direct solar radiation and rain without any protection of its surface will, after a certain period of time, lose its original colour. Moreover, transparent coatings, no matter whether they form films or not, so-called natural wood treatments, do not preserve the shade of the fully exposed wood satisfactorily (H. Kühne, M. Hochweber and J. Sell, 1968).

The nature and the origin of colour changes may have various causes:

1. The colouring matters occurring in many kinds of wood are chemically altered by solar irradiation, which may cause, depending on the type of wood, varying discolourations (W. Sandermann and M. Lüthgens, 1953). Some of these colouring matters are either water soluble, or they are degraded by the influence of the light and consequently become water soluble. They are then leached out by the rain. As a result the wood becomes more and more bleached (W. Sandermann and F. Schlumbom, 1962).
2. As stated above lignin is degraded by solar irradiation in surface close zones. This is first shown by the wood turning yellow, unless this is superimposed by other colour changes. Exposure to artificial ultraviolet (and visible) light proved that most of the light coloured woods turn yellow (G. J. Leary, 1967; W. Sandermann und M. Lüthgens, 1953; W. Sandermann and F. Schlumbom, 1962). As the water soluble reduction products of lignin are leached out by rain, the cellulose content at the surface of the weather exposed wood is finally increased (O. Wälchli, 1957). A. Frey-Wyssling (1950) considered this process the main reason of "natural greying" of light coloured wood, in particular of native conifer timber. However, this author did not exclude the participation of dispersed iron, humidity and certain fungi in the process of greying. Investigations carried out by O. Wälchli (1957) proved that the ultraviolet radiation and the exposure to the weather produce similar degrading effects on wood treated with preservatives (e. g. PCP-containing products). The lignin is destroyed in the external layers, in a zone of approximately 0.2 to 1 mm depth. Consequently there results a zone of enriched pure cellulose. The wood remains free of fungal attack and does not turn grey, but various shades of yellow to shades of a greyish-white can be observed.
3. More recent investigations by O. Wälchli (1957 and 1967) and J. Sell (1968) have proved that the greying of light coloured woods is

practically only due to the growth of blue-staining fungi in the external layers of timber. The most frequently observed species is *Pullularia* (= *Aureobasidium*) *pullulans* (de Bary and Löw) Berkhout. On wood turned grey from avalanche barriers not only *Pullularia pullulans* was found, but also fungi belonging to the genera of *Macrosporium, Tetracoccosporium, Cladosporium, Sclerophoma* and some other Dematiaceae. In the course of the exposure to the weather the cells situated nearest to the surface of the wood are more and more colonized by the fungi. After two years' exposure to the weather with moderate precipitations (northern Switzerland) dark coloured to black mycelium grew through the cells near the surface which may be taken for "pigmentation" (Figures 1 and 2).

The growth of these blue-staining fungi requires at least occasional moistening of the wooden surface by water (precipitation, spray, condensation). Even low annual quantities of precipitations fulfill this requirement, but the intensity and speed of greying increase in proportion to the moisture content.

Experience proves that not only sapwood of pine, spruce, fir and larch trees turns grey, but also, though more slowly, the heartwood of these species. This is also the case with many kinds of broad leaf wood. After a sufficiently long exposure to the influences of the weather, even species of wood which are considered to be fungus resistant are attacked by blue-staining fungi. In the course of the weathering the natural fungicidal substances are leached from the surface of the wood.

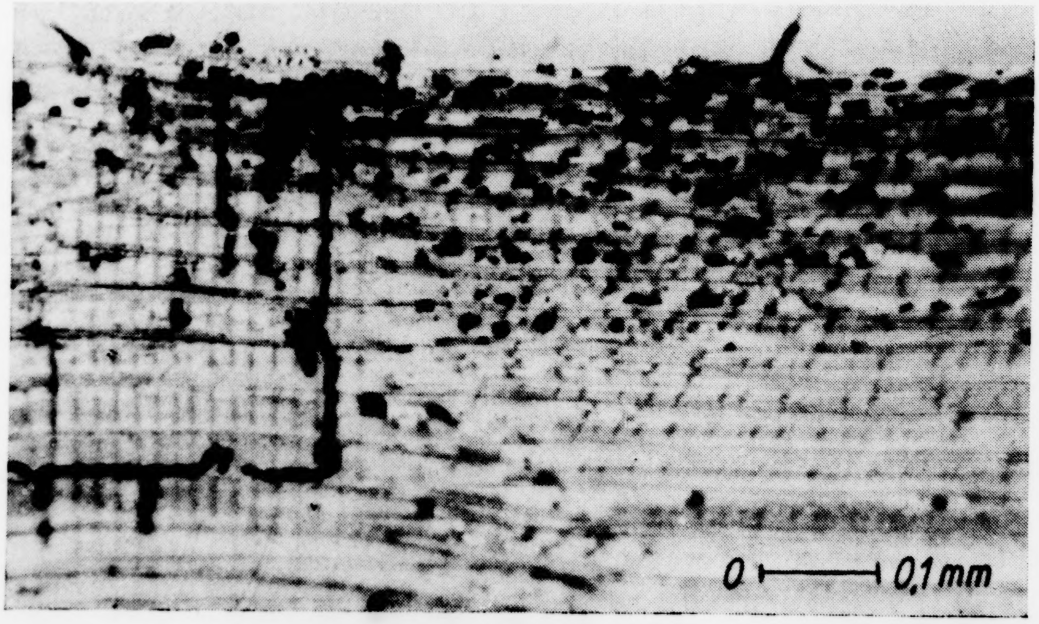


Fig. 1. Radial section through the surface close zone of non-protected spruce board after two years' exposure to the weather. The external layers of cells are strongly penetrated by *Pullularia pullulans*.

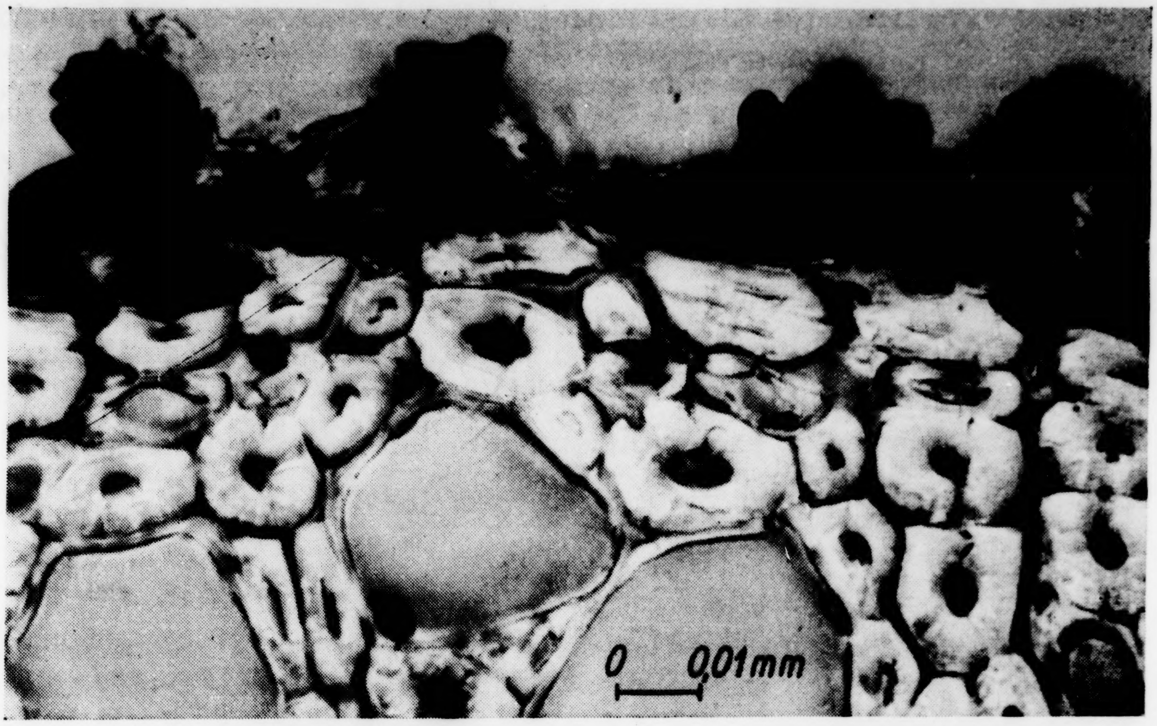


Fig. 2. Cross section through the cells near the surface of a beech board after one year's exposure to the weather. Superficial colonization by *Pullularia pullulans*.

4. Discolouring of timber may also originate from a reaction of substances contained in the wood with impurities of the air and components of the soil etc. The iron-tannin reaction is a well-known reaction which blackens some species of wood (W. Sandermann and M. Lüthgens, 1953).

Of the above mentioned discolouration effects the first three are very often superimposed. Bleaching and the growth of fungi occur simultaneously on light coloured woods. The result is a discolouration of the surface of the wood (solar radiation and rain without the growth of fungi turn the wood nearly white in the course of time). These observations make it plausible why it is not possible to reproduce the greying of light coloured wood under artificial weathering conditions.

Blue-staining fungi are able to grow across thick films of resins and to grow on pigmented paint coatings, provided the pigment itself is not fungicidal (J. Sell, 1968). Other species of fungi can even perforate thin metal foils (W. Liese and R. Schmid, 1964). Moreover, *Pullularia pullulans* is relatively resistant against fungicides.

So-called impregnating primers applied under film-forming coatings did not prove to yield permanent protection against blue-staining fungi (H. Kühne, M. Hochweber and J. Sell, 1968). On the other hand, numerous coating pigments proved to have a fungicidal effect. Therefore, it

is possible to prevent the blue-staining of the surface by the application of appropriate paint coatings.

However the preservation of the original shade of the wood proves to be difficult. Treatments of the original wood with transparent impregnant coatings and colourless varnishes do not fulfill this task if the wood is permanently weather-exposed. (Moreover the renovation with colourless varnishes is quite expensive).

At the moment the most effective way to conserve an aesthetically satisfactory appearance is an adequate architectural and constructive protection against solar radiation and moistening by liquid water (H. KÜHNE, 1966). On the other hand, uniform discolourations might be tolerated.

3. Summary

Substantial technical and aesthetical changes of the surface of weather exposed wood in open air constructions and their causes have been compiled from the literature. In particular, mentions has been made of recent investigations regarding greying, which occurs especially on native conifers (softwood) and which originates from an attack by certain species of bluestaining fungi. On these softwoods and on many coloured woods, sun irradiation alone leads only to bleaching, but not to greying.

Zusammenfassung

Änderungen der Oberflächenbeschaffenheit der Bewitterung ausgesetzten Holzes

Wesentliche technische und ästhetische Veränderungen der Oberflächenbeschaffenheit von freibewittertem Holz und ihre Ursachen wurden aus der Literatur zuasmmengestellt. Besonders erwähnt wurden neuere Untersuchungen, wonach die Graufärbung, welche besonders bei hellen einheimischen Nadelhölzern auffällt, auf eine Besiedlung durch bestimmte Bläuepilze zurückzuführen ist. Die Sonneneinstrahlung allein verursacht bei diesen und auch bei vielen farbigen Hölzern lediglich eine Ausbleichung, nicht aber eine Vergrauung.

Résumé

Altérations de la texture superficielle du bois exposé aux intempéries

Les altérations techniques et esthétiques substantielles de la texture superficielle du bois exposé aux intempéries et leurs causes ont été compilées de la littérature. En particulier mention est faite des investigations récentes, desquelles il s'avère que le grisonnement, qui se manifeste principalement sur les bois clairs des conifères indigènes, est provoqué par certains champignons bleuissants. L'insolation seule ne provoque qu'un blanchiment de ces bois et aussi d'autres bois colorés, mais jamais un grisonnement.

Resumen

Cambios en la textura superficial de maderas expuestas a la intemperie

Las alteraciones técnicas y estéticas substanciales de la textura superficial de las maderas se han copilado de la literatura. Se ha hecho especial mención

a los trabajos recientes de investigación acerca de las coloraciones grisáceas, que se manifiestan principalmente sobre coníferas nativas (maderas blandas) y las cuales son provocadas por el ataque de ciertos hongos causantes de la mancha azul. La sola irradiación solar no produce para estas maderas blandas, lo mismo que para muchas otras coloreadas, más que un blanqueamiento, pero nunca coloraciones grisáceas.

Literature

Frey-Wyssling, A.: Die Verfärbung ungeschützten Holzes durch das Wetter. Schweiz. Z. f. d. Forstwes. *101* (1950) 278—282.

Keylwerth, R.: Temperatur- und Feuchtigkeitsgefälle in Holzbauteilen. Holz als Roh- u. Werkstoff *24* (1966) No. 10, 452—454.

Kühne, H.: Holzschutz als Aufgabe des projektierenden Baufachmannes. Schweiz. Bauztg. *84* (1966) No. 5, 99—106.

Kühne, H., Hochweber, M. and Sell, J.: Freiland-Bewitterungsversuche an Außenanstrichen für Holz — Versuchszeitraum 1962 bis 1967. EMPA-Bericht Nr. 182 Dübendorf, 1968.

Leary, G. J.: The yellowing of wood by light. Tappi *50* (1967) No. 1, 17—19.

Liese, W. and Schmid, R.: Über das Wachstum von Bläuepilzen durch verholzte Zellwände. Phytopath. Z. *51* (1964) No. 4, 385—393.

Miniutti, V. P.: Microscopic observations of ultraviolet irradiated and weathered softwood surfaces on clear coatings. U. S. Forest Service, Research Paper FPL 74, May 1967.

Sandermann, W. and Lüthgens, M.: Untersuchungen über Verfärbungen von Hölzern. Holz als Roh- u. Werkstoff *11* (1953) No. 11, 435—440.

Sandermann, W. and Schlumbom, F.: Über die Wirkung gefilterten ultravioletten Lichtes auf Holz. 1. Mitt.: Photometrische und chromatographische Untersuchungen an Holzmehlen. 2. Mitt.: Änderungen von Farbwert und Farbempfindung an Holzoberflächen. Holz als Roh- u. Werkstoff *20* (1962) No. 7, 245—252 and No. 8, 285—291.

Sell, J.: Untersuchungen über die Besiedlung von unbehandeltem und angestrichenem Holz durch Bläuepilze. Holz als Roh- u. Werkstoff *26* (1968) No. 6, 215—222.

Wälchli, O.: Unveröffentlichte Untersuchungsergebnisse aus Untersuchungsbericht VII/1829 der EMPA, Eidg. Materialprüfungs- und Versuchsanstalt, St. Gallen vom 6. Juli 1957 sowie von Untersuchungen aus dem Jahr 1967.

Addresses of the authors:

Dipl.-Holzwirt Jürgen Sell
EMPA Dübendorf
Abt. Holz und Kunststoffe
8600 Dübendorf, Schweiz

Dr. Oskar Wälchli
EMPA St. Gallen
Abt. Werkstoff-Biologie
Unterstraße 11
9001 St. Gallen, Schweiz

LENZ, von M. 1969

Zur schädigenden Wirkung einiger Schimmelpilze auf Termiten.

- Mater. & Org., 4 (2): 109-122

[6 fig., 1 table. Engl., Fr., Span. summ.]

Microfilm No. 15627

Zur schädigenden Wirkung einiger Schimmelpilze auf Termiten

Von MICHAEL LENZ

Bundesanstalt für Materialprüfung Berlin-Dahlem, Fachgruppe „Biologische Materialprüfung“[1]

Inhalt

1. Einleitung
2. Durchführung der Versuche
3. Toxische Wirkung des Kulturfiltrats einiger Schimmelpilze
4. Einfluß des Alters von Pilzkulturen auf ihre toxische Wirkung
   4.1 Versuche mit Kulturfiltraten — 4.2 Versuche mit Agarkulturen
5. Zusammenfassung

Schrifttum

1. Einleitung

Der Lebensraum „Holz“ wird von verschiedenen Tieren und Mikroorganismen gleichermaßen bewohnt. Beide Organismengruppen stehen in vielseitigen Wechselbeziehungen zueinander (G. BECKER, 1950, 1966 a; K. GRAHAM, 1967). Kompliziert werden diese dadurch, daß man nicht allgemein einer Mikroorganismenart eine bestimmte Bedeutung — fördernd, indifferent, schädigend — für die Tiere zuschreiben kann; denn einschränkend muß stets vermerkt werden: nur der jeweils untersuchte Stamm der Mikroorganismen übt die festgestellte Wirkung aus (G. BECKER und W. KERNER-GANG, 1964; M. LENZ, 1968).

Für Termiten wird in der Literatur auf vielseitige Beziehungen zu Pilzen verwiesen. Bisher haben aber nur G. BECKER und W. KERNER-GANG (1964) und G. BECKER (1965 b) systematisch Schimmelpilze und Braunfäuleerreger in ihrer Bedeutung als Nahrung und ihren Einfluß auf die Entwicklung für verschiedene Termitenarten geprüft. In der vorliegenden Arbeit wurde die Toxinwirkung eines Stammes von *Aspergillus niger* auf Termiten untersucht. Zu Vergleichszwecken sind in einigen Fällen auch noch andere Pilze, von denen Toxinbildung be-

---

[1] Die Arbeit wurde durch eine Prof. Dr. GÜNTHER BECKER von der Deutschen Forschungsgemeinschaft gewährte Förderung ermöglicht. Diesem danke ich für Anregungen und Diskussion.

kannt war, mit herangezogen worden. Außer der Wirkung des Kulturfiltrats wurde verfolgt, welchen Einfluß das Alter des Pilzes auf seine Wirkung Termiten gegenüber hat.

Die Bildung toxischer Stoffe durch Mikroorganismen, besonders durch Schimmelpilze, wird ganz allgemein zu einem immer mehr beachteten Problem. Im Bereich der menschlichen Ernährung, der Tierzucht u. a. gilt es, das Auftreten von Pilzen auf der Nahrung, dem Tierfutter und anderen Stoffen überhaupt zu verhindern bzw. Verfahren zum Nachweis und zur Ausschaltung der Gifte auszuarbeiten.

Andererseits können derartige Toxinbildner im Rahmen der biologischen Schädlingsbekämpfung u. U. nutzbringend eingesetzt werden. In jedem Falle interessiert, wie die Toxine auf die Tiere einwirken, und das Verhalten der Tiere, wenn sie mit entsprechenden Mikroorganismen in Berührung kommen. Derartige Fragen wurden bisher überwiegend an Wirbeltieren untersucht. Relativ wenig Material liegt über andere Tiergruppen vor (E. Müller-Kögler, 1965; M. Lenz, 1968).

2. Durchführung der Versuche[2]

Versuchstiere waren die beiden Rhinotermitiden *Heterotermes indicola* (Wasmann) aus Nord-Indien und *Coptotermes amanii* (Sjöstedt) aus Ost-Afrika. Beide Zuchten werden in der Bundesanstalt für Materialprüfung in Berlin-Dahlem seit Jahren bei 30° C gehalten (G. Becker, 1965 a, 1966 b). Diese Arten wurden ausgewählt, da sie stets in gleichbleibend großer Zahl zur Verfügung standen. Für einleitende Versuche wurde noch mit der Trockenholztermite *Kalotermes flavicollis* (Fabr.) aus Süd-Italien gearbeitet.

Die benutzten Pilze waren[3]:

Aspergillus flavus IMI 89 717 und T 14
Aspergillus niger ATCC 6275
Gliocladium virens MG 10
Myrothecium verrucaria OECD 6

Die Pilze wurden zur Gewinnung von Kulturfiltraten in Czapek-Dox-Lösung gezogen. In allen anderen Fällen diente Malzagar als Nährmedium (vgl. M. Lenz, 1968, S. 310 u. 385).

Filtrate oder Extrakte wurden zur Verfütterung an die Termiten auf Filtrierpapierscheibchen von 18 mm ⌀ getropft (0,2 ml). Die Proben wurden 2 Tage im Exciccator aufbewahrt, anschließend gewogen und für die Dauer von 48 Stunden den Tieren in Glasschalen angeboten.

3. Toxische Wirkung des Kulturfiltrats einiger Schimmelpilze

Filtrat von 11tägigen Kulturen der fünf verwendeten Schimmelpilze wurde an je 10 Tiere (5 Larven, 5 Nymphen) von *Kalotermes flavi-*

---

[2] Für vielfältige Hilfe bei der Durchführung der Versuche danke ich Frl. S. Dietz und Herrn E. von Seydlitz-Kurzbach.
[3] Für Überlassung der Pilzkulturen sei Frau Dr. W. Kerner-Gang und Frl. S. Briese auch an dieser Stelle gedankt.

collis, an 20 Arbeiter von *Heterotermes indicola* und an 15 Arbeiter mit 5 Soldaten von *Coptotermes amanii verfüttert*. Die Tiere befanden sich in Glasschalen von 5 cm ⌀; die Versuchstemperatur betrug bei *Kalotermes* 26° C, bei den anderen Arten 30° C. Zur Einstellung der nötigen Luftfeuchtigkeit erhielten die beiden Rhinotermitiden einen Streifen feuchten Filtrierpapiers am Schalendeckel. Für jeden Pilz wurden zwei Ansätze hergestellt; je Ansatz wurden 5 Papierscheibchen getränkt.

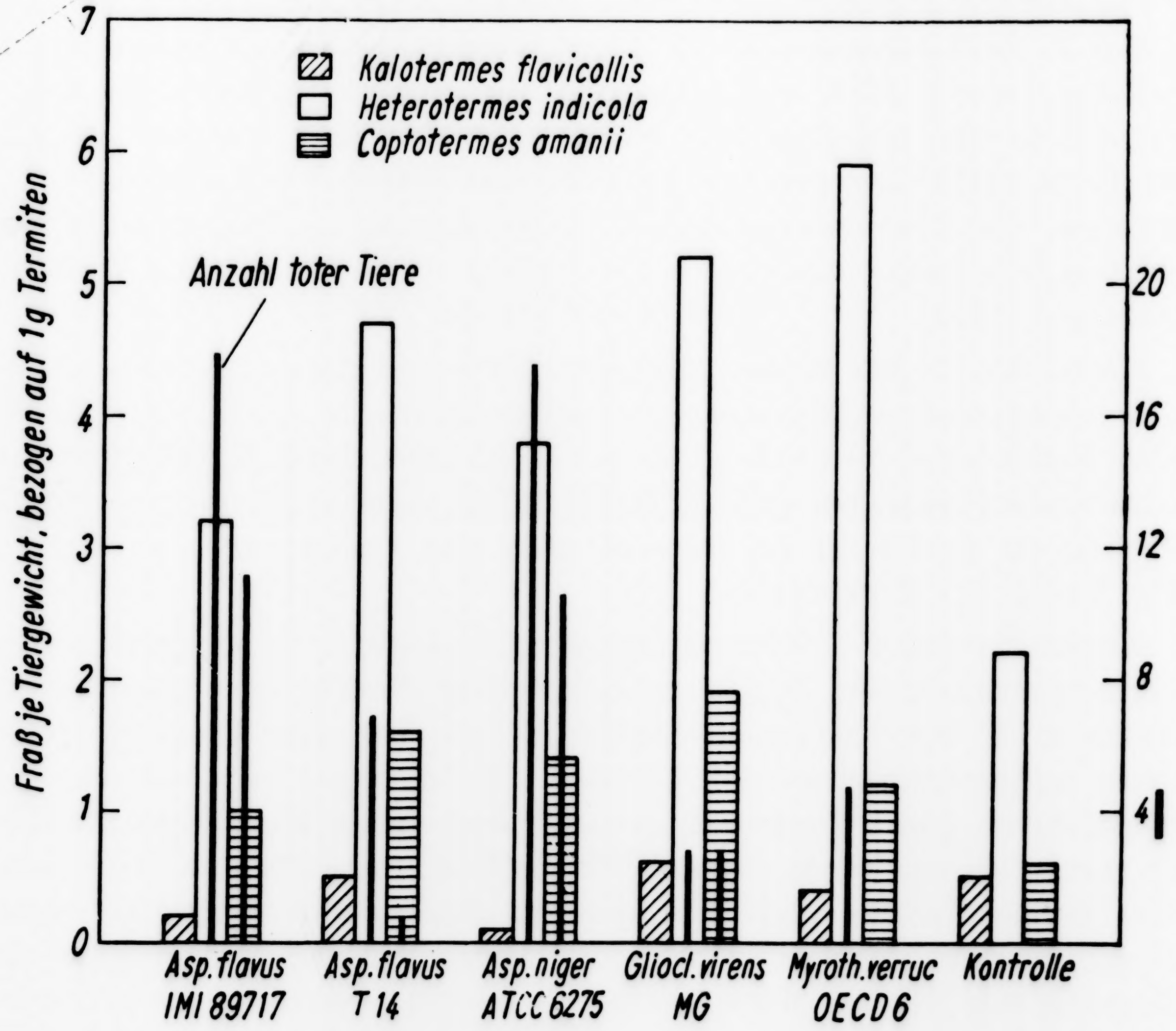


Fig. 1. Verfütterung von 11tägigem Kulturfiltrat einiger Schimmelpilze an 3 Termitenarten. Fraß (Ordinate links) und Anzahl der toten Tiere (Ordinate rechts) wurden nach 48 Stunden bestimmt.

Kalotermes frißt, wie Fig. 1 zeigt, von allen drei Termitenarten am wenigsten, *Heterotermes* ist am gefräßigsten. Die nur mit Wasser getränkte Kontrolle wird vergleichsweise wenig angenommen; dafür überstehen die Tiere den Fraß ohne Schaden. Die Proben aller 5 Schimmelarten schädigen *Heterotermes*, und zwar unterschiedlich stark; *Coptotermes* reagiert weniger empfindlich, aber nur ein Pilz, ein

Stamm von *Myrothecium verrucaria*, bleibt ganz ohne Wirkung auf diese Art. Die höchste Sterberate wird bei den beiden Rhinotermitiden übereinstimmend von dem *Aspergillus flavus*-Stamm IMI 89 717 und dem *A. niger*-Stamm ATCC 6275 erzielt. Beide Pilze wirken etwa gleich stark giftig. *Kalotermes*-Individuen starben in keinem Fall, fraßen aber doch die Filtrate der beiden genannten Pilze nur wenig. *Kalotermes* schied somit als Versuchstier für eine Prüfung der Schadwirkung von Schimmelpilzen aus. Diese Art erweist sich überhaupt als sehr robust und weicht in ihrem Verhalten oft von dem anderer Termitenarten ab (G. Becker, 1961, 1962).

Der *A. flavus*-Stamm IMI 89 717 ist nach I. Elphick als Toxinbildner bekannt (briefl. Mitt. an G. Becker). Ein Nematode, *Rhabditis* spec., vermehrte sich auf dem Pilz kaum, auch der Fraß war nur gering (M. Lenz, 1968). Gruppen von *Nasutitermes ephratae* und *Heterotermes indicola*, die den verwendeten *A. niger*-Stamm als Futter erhielten, starben innerhalb eines kurzen Zeitraumes ab (G. Becker und W. Kerner-Gang, 1964).

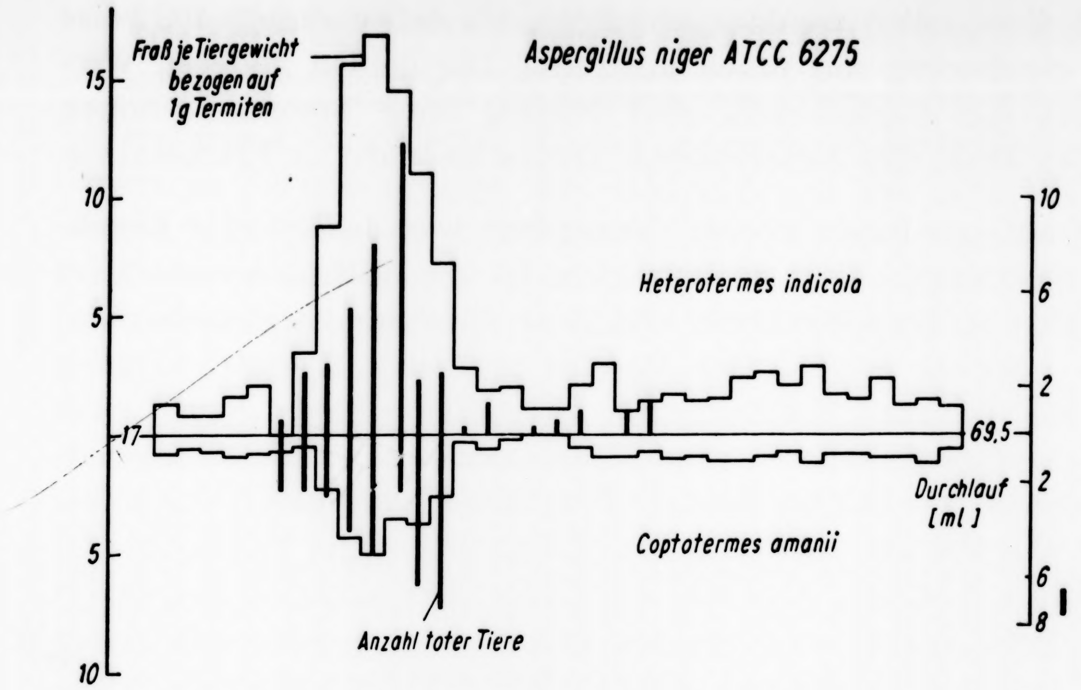
Kurturfiltrate der beiden giftigsten Pilze und des nur wenig schädlichen Stammes von *Myrothecium verrucaria* wurde anschließend durch eine Sephadex-G-10-Säule geschickt (s. M. Lenz, 1968, S. 400) und die erhaltenen Fraktionen (bei *A. flavus* 31, *A. niger* 35, *M. verrucaria* 43 Proben zu je 1,5 ml) an *Heterotermes* und *Coptotermes* verfüttert. Fig. 2 zeigt die Ergebnisse.

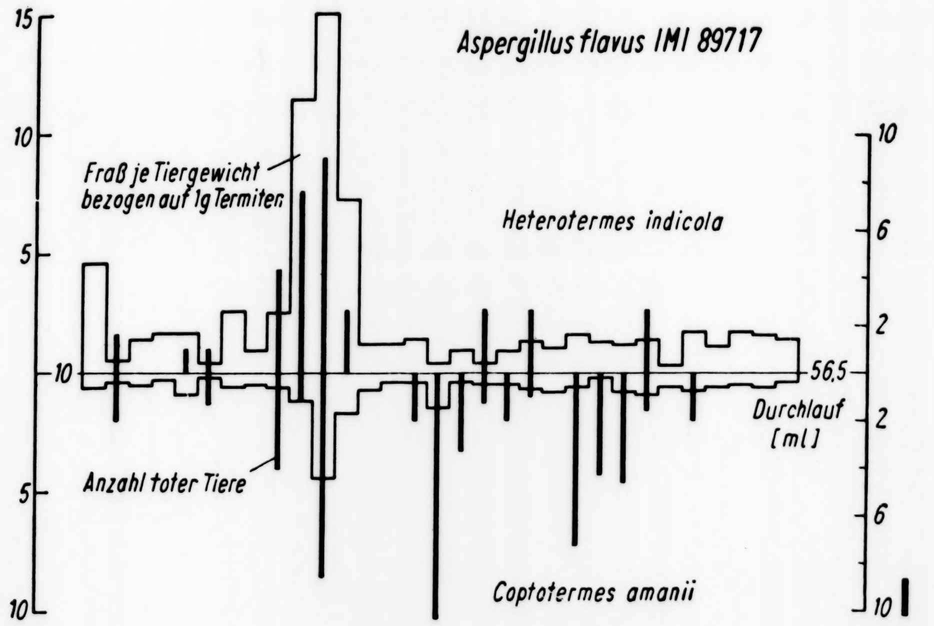
Allgemein fallen Fraßmaximum und höchste Zahl an gestorbenen Termiten zusammen. In den entsprechenden Fraktionierungsbereichen traten z. T. hygroskopische Stoffe auf, die von den Termiten recht leicht aufgenommen werden konnten. Für *A. niger* hebt sich ein für beide Arten gleich liegendes, deutlich begrenztes Wirkungsmaximum ab. Lediglich die gegen diesen Pilz empfindlichere Termite *Heterotermes indicola* wurde noch von späteren Fraktionen geschädigt, wenn auch nur geringfügig. Für beide Termitenarten decken sich zwar die Bereiche mit lethaler Wirkung, jedoch liegt das Maximum bei *Heterotermes* etwas vor dem von *Coptotermes*.

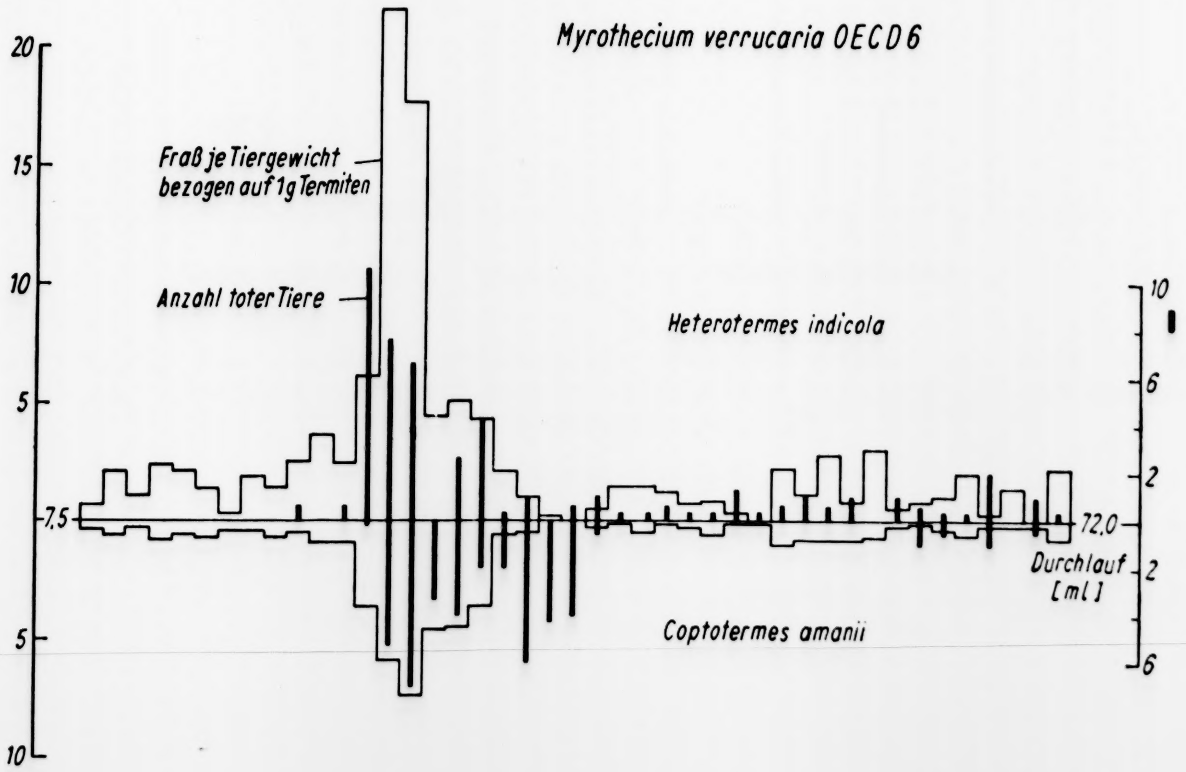
Beim *A. flavus*-Stamm ist das Bild differenzierter. *Coptotermes* reagiert recht empfindlich auf diesen Pilz, *Heterotermes* weniger. Erstere Art wird an drei Stellen innerhalb des Fraktionierungsbereiches stark geschädigt. In der Lage des ersten Maximums stimmen beide Arten überein. Eine Schädigung geht hier schon von einer Fraktion aus, die noch nicht hygroskopische Stoffe enthielt. Das ist wohl als Hinweis zu

---

Fig. 2. Wirkung der einzelnen Proben eines mit Hilfe einer Sephadex-G-10-Säule fraktionierten Alkoholextraktes aus dem Kulturfiltrat von *Aspergillus niger* ATCC 6275, *A. flavus* IMI 89 717 und *Myrothecium verrucaria* OECD 6 auf *Heterotermes indicola* und *Coptotermes amanii*.


Aspergillus niger ATCC 6275
Fraß je Tiergewicht bezogen auf 1g Termiten
Heterotermes indicola
Coptotermes amanii
Anzahl toter Tiere
Durchlauf [ml]
17
69,5
15
10
5
10
5
10
6
2
2
6
8


Aspergillus flavus IMI 89717
Fraß je Tiergewicht bezogen auf 1g Termiten
Heterotermes indicola
Coptotermes amanii
Anzahl toter Tiere
Durchlauf [ml]
10
56,5
15
10
5
5
10
10
6
2
2
6
10


Myrothecium verrucaria OECD 6
Fraß je Tiergewicht bezogen auf 1g Termiten
Anzahl toter Tiere
Heterotermes indicola
Coptotermes amanii
Durchlauf [ml]
7,5
72,0
20
15
10
5
5
10
10
6
2
2
6

werten, daß diese selbst weniger schädigen, als daß vielmehr teilweise die Toxine gleichzeitig mit ihnen auftreten. Die beiden anderen Wirkungsmaxima für *Coptotermes* sind Stellen ohne besonders hohen oder geringen Fraß. Bei *Heterotermes* treten sie nicht in Erscheinung.

Nach den anfangs beschriebenen Versuchen war das erzielte Ergebnis mit *M. verrucaria* nicht zu erwarten. Ist das Filtrat konzentriert genug, schädigt es Termiten doch, und zwar durchaus vergleichbar mit den beiden anderen Pilzen. Der Bereich stärkerer Beeinträchtigung deckt sich für die zwei Termitenarten nicht ganz. Beide zeigen zwei nur wenig auseinanderliegende Gipfel. *Heterotermes* wird sehr schwach auch noch durch die anschließenden Fraktionen geschädigt, *Coptotermes* kaum. Wurden aus *M. verrucaria*-Kulturfiltrat und Pilzmycel mit Sporen wäßrige Auszüge, Alkohol- und Chloroformextrakte hergestellt (s. M. Lenz, 1968, S. 391), schadete den Termiten keine der verfütterten Lösungen (jeweils 5 Parallelen). Alle Proben wurden gefressen, wenn auch mit gewissen bei beiden Arten annähernd übereinstimmenden Unterschieden (Fig. 3). Kulturfiltrat wurde besser angenommen als der Pilz, am stärksten der wäßrige Auszug, am wenigsten der Chloroformextrakt.

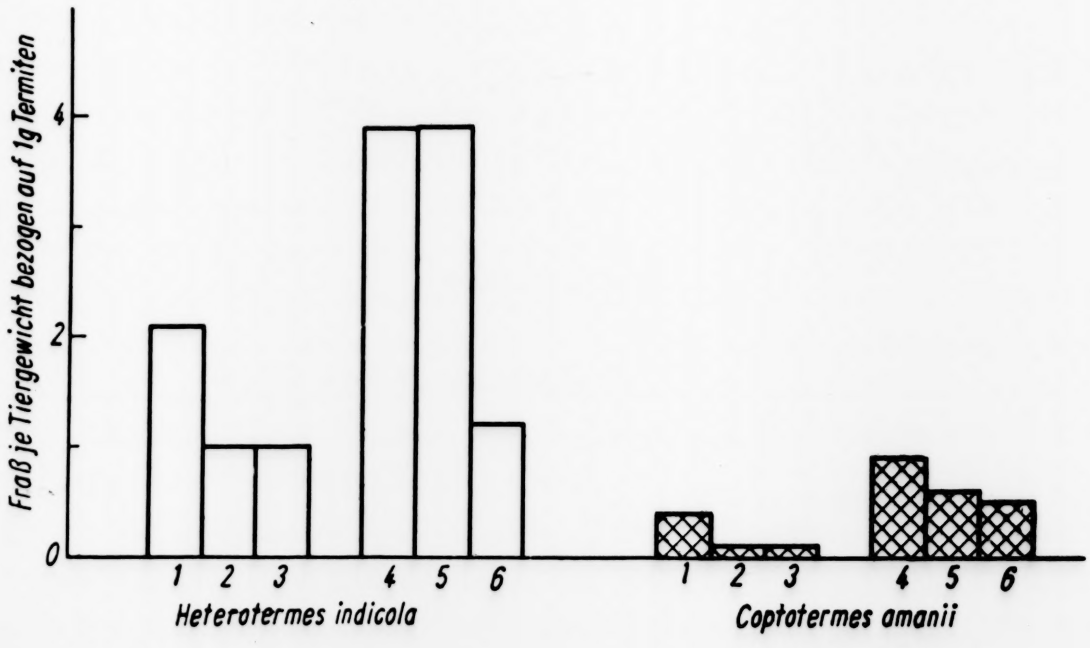


Fig. 3. Fraß der beiden Termiten *Heterotermes indicola* und *Coptotermes amanii* bei Verfütterung von Extrakten aus 12tägigen Kulturen von *Myrothecium verrucaria* OECD 6.

1 Wasser- 2 Alkohol- 3 Chloroform- auszug	Pilzmycel und -sporen	4 Filtrat 5 Alkohol- 6 Chloroform- auszug	Nährlösung

4. Einfluß des Alters von Pilzkulturen auf ihre toxische Wirkung

Bei der Prüfung des Verhaltens von Tieren gegenüber Schimmelpilzen wie auch anderen Mikroorganismen ist eine Vielzahl von Faktoren zu berücksichtigen. Ein wichtiger ist das Alter des Pilzes. So treten z. B. Giftstoffe oft während einer bestimmten Wachstumsphase auf oder erreichen erst dann eine wirksame Konzentration; andererseits können solche Stoffe im Laufe der Zeit auch wieder verschwinden.

Wurden Kellerasseln (*Porcellio scaber* Latreille) und Collembolen (*Entomobrya marginata* Tullberg) auf 24 und 48 Stunden alten *Aspergillus niger* ATCC 6275 gesetzt, starben die Tiere auf dem älteren Pilz deutlich früher als auf der nur 24 Stunden jüngeren Kultur. Offensichtlich werden in dieser kurzen Zeitspanne physiologische Prozesse eingeleitet, die den Pilz für die Tiere weniger verträglich machen. Der entscheidende Vorgang dürfte die Ausbildung der Konidien sein. Die Sporulation setzt auf festem Nährboden zwischen dem ersten und zweiten Tag des Pilzwachstums ein, in flüssiger Kultur später. Im Zusammenhang damit oder paralell zur Sporulation kommt es zur Bildung

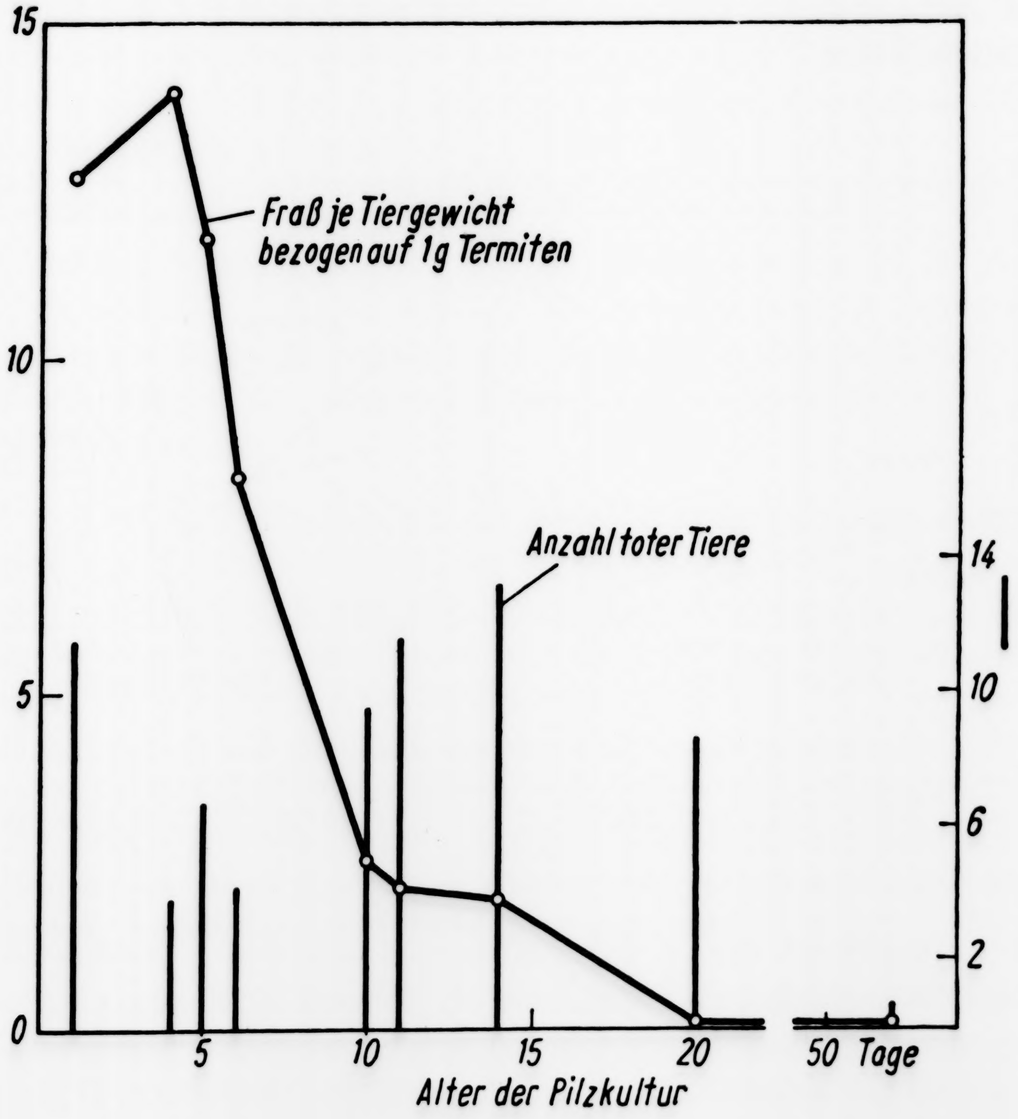


Fig. 4. Fraß und Anzahl toter Termiten von *Heterotermes indicola* und *Coptotermes amanii* bei Verfütterung von Kulturfiltrat von *Aspergillus niger* ATCC 6275-Kulturen verschiedenen Alters.

von Toxin(en)[4], wie durch einen Vergleich der Wirkung des Kulturfiltrates von sporuliertem (Standkultur) und sporenfreiem Ansatz (Schüttelkultur) auf die Termite *Heterotermes indicola* gezeigt werden konnte. Ältere Kulturen dagegen werden wieder leichter angenommen. So fraß *Heterotermes* Extrakte eines 7wöchigen *Aspergillus flavus*-Stammes besser als die einer 11tägigen Kultur (M. Lenz, 1968).

4.1 Versuche mit Kulturfiltraten

Aus Kulturen von *A. niger* ATCC 6275 wurde nach 3, 4, 5, 6, 10, 11, 14, 20 und 52 Tagen Filtrat entnommen und an *Heterotermes* verfüttert. Es wurden 3 Pilzansätze mit je 5 Tierparallelen geprüft; die in Fig. 4 dargestellten Ergebnisse sind das Mittel aus 15 Werten. Mit zunehmendem Alter des Pilzes nimmt der Fraß ab. Die Giftwirkung bleibt bis zum 20. Tag, an dem zugleich der Fraß seinen niedrigsten Wert erreicht, voll erhalten. Ist die Kultur 7 Wochen alt, ist sie zwar für die Termiten nicht „bekömmlicher" geworden, immerhin sind aber Toxine nur noch in geringer Konzentration vorhanden. Es sterben kaum noch Tiere.

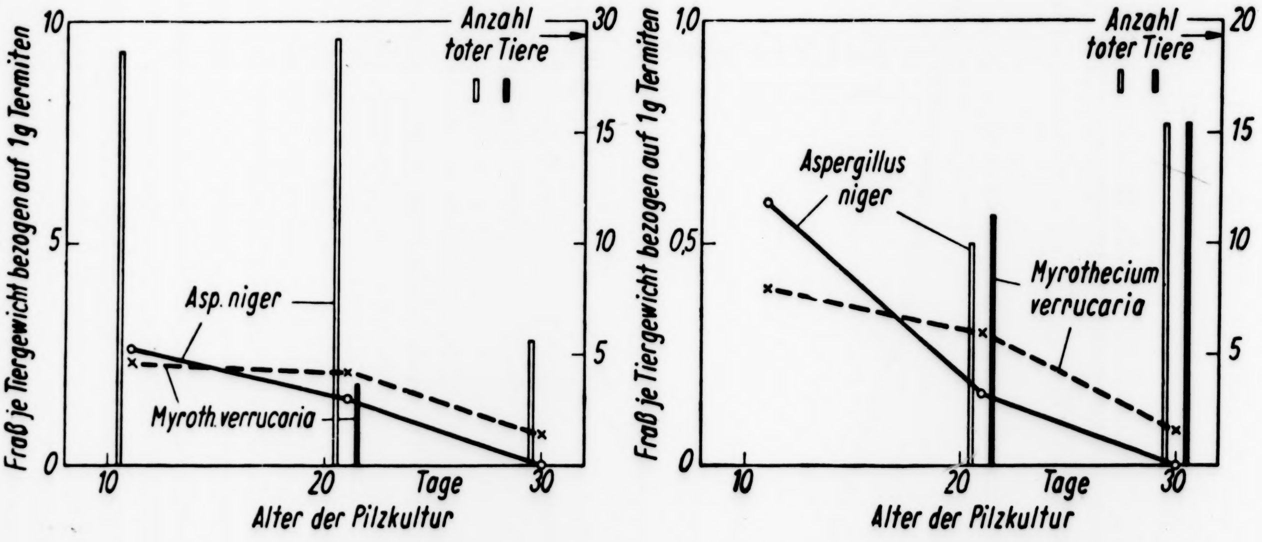


Fig. 5. Fraß und Anzahl toter Termiten von *Heterotermes indicola* (links) und *Coptotermes amanii* (rechts) bei Verfütterung von Kulturfiltrat von *Aspergillus niger* ATCC 6275 und *Myrothecium verrucaria* OECD 6 verschiedenen Alters.

Auf Kulturfiltrat des *A. niger*-Stammes und von *Myrothecium verrucaria* OECD 6, das im Alter von 11, 21 und 30 Tagen vergleichend an *Coptotermes* und *Heterotermes* verfüttert wurde, reagierten die Tiere in Übereinstimmung mit den anderen Versuchen (Fig. 5). Mit

[4] Bei dem (den) toxischen Stoff(en) dieses Stammes von *A. niger* als auch der anderen geprüften Stämme handelt es sich nicht um Aflatoxin (H. K. Frank briefl. an G. Becker, 1969).

zunehmendem Alter werden die Pilze weniger gefressen. Für *Heterotermes* ist *A. niger* toxisch, 30 Tage alt allerdings weniger als vorher. Bei *Myrothecium* schädigt nur die 21 Tage alte Kultur etwas. *Coptotermes* wird von beiden Pilzen im Alter von 21 und 30 Tagen in etwa gleich hohem Maße beinträchtigt. Im Gegensatz zu anderen Befunden starben durch 11tägiges *A. niger*-Filtrat bei dieser Art keine Tiere.

Aus diesen Versuchen kann allgemein geschlossen werden, daß die Toxinbildung bei den untersuchten Pilzen innerhalb der ersten 3 (bis 4) Wochen des Pilzwachstums ihr Maximum hat; danach fällt die Konzentration an für Termiten schädlichen Stoffen offensichtlich schnell ab.

4.2 Versuche mit Agarkulturen

Für die Beurteilung eines möglichen günstigen oder schädigenden Einflusses von holzzerstörenden Pilzen auf *Reticulitermes* hat A. E. Lund (1960) die Termiten direkt in die mit dem entsprechenden Pilz bewachsenen Schalen gesetzt. Lebensdauer und Zahl und Ausmaß der Gänge, die die Tiere im Nährboden anlegten, dienten als Maßstab für die Wirkung der Pilze.

In Anlehnung an dieses Verfahren und entsprechend, wie schon früher mit anderen Tierarten durchgeführt, wurden je 20 Arbeiter von *Heterotermes indicola* und je 20 Arbeiter und 5 Soldaten von *Coptotermes amanii* zu Schalen von 9 cm ∅, in denen Schimmelpilze für 24 oder 48 Stunden lang auf Malzagar gewachsen waren, in jeweils 5 Parallelen gesetzt.

In Tab. 1 sind die Ergebnisse aus zwei derartigen Reihen dargestellt. Angegeben ist jeweils die Zeit in Tagen, nach der 50 % der Tiere gestorben waren (LT_{50}). Wenn sich auch allgemein abzeichnet, wie schon anfangs erwähnt, daß die Tiere auf dem 2 Tage alten Pilz rascher absterben als auf dem nur 24 Stunden jüngeren, zeigt ein näherer Vergleich, daß die Werte z. T. stark streuen, besonders bei *Myrothecium verrucaria*. Die Unterschiede sind teilweise auf nicht ganz einheitliche Ausgangsbedingungen zurückzuführen. Hatten die Tiere pilzfreie Stellen auf dem Agar oder solche mit nur schwach entwickeltem Mycelrasen, hielten sie sich bevorzugt dort auf.

Von diesen Flächen aus fraßen sie Löcher und Gänge in den Nährboden, aber nur wenig vom Pilz. Dieser drang oft in die Gänge vor, zumal die Termiten die Sporen verschleppten. Waren solche für einen Aufenthalt günstigen Stellen nicht vorhanden, liefen die Tiere hauptsächlich am Schalenrand entlang und hielten sich oft sogar an der Schalenwand auf.

Tab. 1. Zeit in Tagen, nach der 50 % der Tiere von je 20 Arbeitern von *Heterotermes indicola* und von je 20 Arbeitern und 5 Soldaten von *Coptotermes amanii* auf 24 und 48 Stunden alten Agarkulturen von Schimmelpilzen in Schalen von 9 cm ⌀ starben (LT_{50}).

Reihe	Pilz[a]	LT_{50} in Tagen					
		Heterotermes indicola Pilzalter (Std.)		*Coptotermes amanii* Pilzalter (Std.)			
		24	48	24		48	
				Arb.	Sold.	Arb.	Sold.
1	*Asp. flavus* IMI 89 717	3,2	1,0	4,3	3,5	3,3	2,7
	Asp. niger	8,4	5,1	20,0	7,5	2,5	2,1
	Myr. verruc.	5,3	5,4	5,6	4,3	11,9	4,0
	Kontrolle (ohne Pilze)	5,6	5,6	21,5	8,9	21,5	8,9
2	*Asp. flavus* IMI 89 717	4,1	3,3	4,8	4,7	2,4	3,5
	Asp. niger	4,3	3,8	10,5	8,0	3,8	4,2
	Myr. verruc.	3,3	1,5	3,5	4,3	3,1	3,5
	Kontrolle (ohne Pilze)	4,7	4,7	11,9	8,3	11,9	8,3

a) Stamm siehe S. 110.

Für eine erfolgreiche Prüfung der Pilzwirkung in dieser Form müssen Schalendurchmesser und Nährbodenhöhe möglichst klein gewählt werden, so daß die Tiere sowohl eine Fläche vorfinden, die sie auch abfressen können, ohne daß der Pilz wieder schneller nachwächst, als auch sich nicht tief in den Nährboden einfressen können, und so tatsächlich mit dem Pilz in Berührung kommen und bleiben. Dann sind Aussagen selbst mit kleiner Tierzahl möglich. Noch auf Schalen von nur 5 cm ⌀ blieben die Ergebnisse unbefriedigend. Schließlich wurden Glasröhrchen von 5 cm Höhe und 2 cm ⌀ verwendet. Malzagar wurde 0,5 cm hoch eingegossen und mit *A. niger* ATCC 6275 beimpft (mit Q-Tips). Die Röhrchen wurden bei 30° C und 98 % r.Lf. aufbewahrt. Bei einem Alter der Ansätze von 24, 26, 28 ... 48 Stunden wurden je 20 Arbeiter von *Heterotermes indicola* zugegeben (für jede Altersstufe 15 Paralellen). Die 24 und 26 Stunden alten Proben zeigten nur eine Myceldecke, die 28stündigen die ersten Konidien.

Die LT_{50} beträgt bis zu einem Alter der Pilzkultur von 34 Stunden über 5 Wochen (Fig. 6), maximal bei 28 Stunden altem Ansatz sogar 7 Wochen. Die Werte für 24 und 26 Stunden alten Pilz und für die

Kontrolle nur mit Agar liegen niedriger. Wahrscheinlich stellen der Nährboden allein und das Substrat mit noch sehr jungem Mycel eine weniger gute Nahrungsquelle dar.

Noch bei einem Alter des Pilzes von 34 Stunden zeigen die Tiere eine große Aktivität. Sie fressen Löcher und Gänge in den Nährboden und bauen Galerien aus Malzagar an den Gefäßwänden. Vielfach wurde zuerst eine Galerie im Kreis unmittelbar über dem Nährboden angelegt, von dort aus Gänge an der Wand der Glasröhrchen hoch, z. T. bis zum Wattestopfen und schließlich noch im Kreis am Stopfen entlang. Die Kontrollen wurden zwar stark zerfressen, jedoch wurden kaum deutliche Galerien gebaut; die Wand wurde lediglich im unteren Teil mit Agarpartikeln bedeckt. Der Pilzbewuchs stimulierte also die Galeriebautätigkeit — vielleicht als Ausweichreaktion.

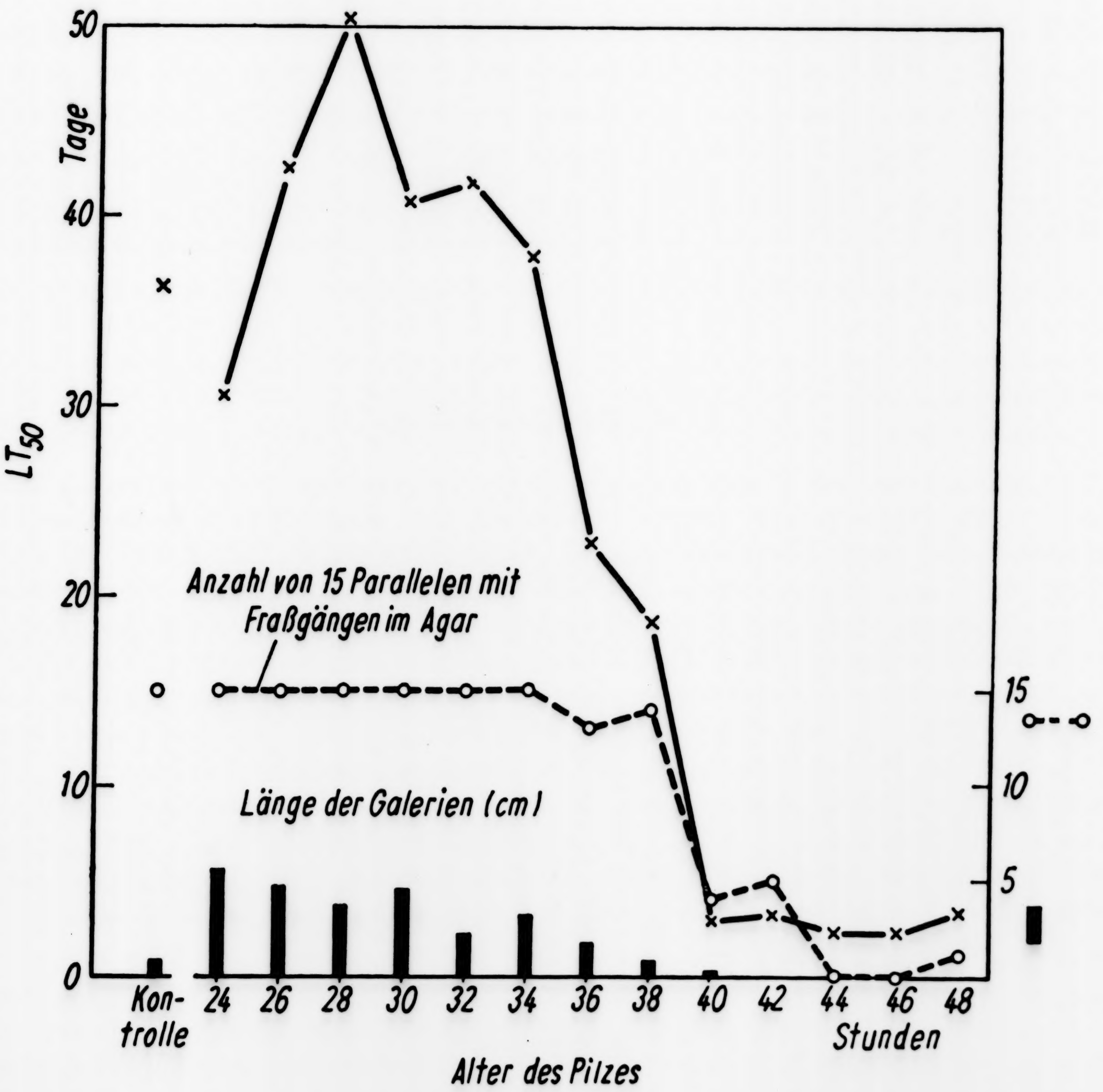


Fig. 6. Lebensdauer (LT_{50}), Anzahl der Parallelen mit Fraßgängen im Agar und Länge der Galerien von *Heterotermes indicola* auf Malzagar in Abhängigkeit vom Alter des daraufwachsenden Pilzes *Aspergillus niger* ATCC 6275.

Bis zu dem angegebenen Pilzalter konnten die Tiere offensichtlich durch ihre Fraßtätigkeit das volle Ausmaß der Sporulation auf der Myceldecke einschränken und damit zugleich die Toxinbildung. Aber bereits von 38 auf 40 Stunden nimmt die Aktivität der Tiere stark ab, die Lebensdauer sinkt bereits im Zeitintervall von 34 zu 36 Stunden, und nochmals sehr von 38 auf 40 Stunden. Die Tiere halten sich dann auf dem Pilz fast nur noch zum Rand der Gefäße zu auf. Sie bauen keine Galerien mehr und in den Agar werden lediglich in Einzelfällen Löcher gefressen. Die LT_{50} beträgt nur noch 3 Tage und weniger.

Offensichtlich steht das Einsetzen der Giftwirkung in Beziehung zur Ausbildung und Reifung der Konidien. Auf diesen zeitlich-physiologischen Zusammenhang ist bisher, obwohl bereits zahlreiche Toxine von Schimmelpilzen bekannt sind, kaum geachtet worden. Entsprechende Zusammenhänge sind bisher nur bei Bakterien untersucht (P. Schaeffer, 1969).

Bei der benutzten Versuchsanordnung hatten die Termiten infolge der kleinen Nährbodenoberfläche in den Glasröhrchen und der geringen Schichthöhe des Agars praktisch keine Gelegenheit, dem Pilz auszuweichen. Wegen der Fernwirkung des Pilzes durch Diffusion von Toxinen in den Nährboden (H. Frank, 1968) scheidet dieser mit zunehmendem Alter des Pilzes als Nahrung für die Termiten aus. Die Versuchsbedingungen entsprachen also in etwa einem Fraßzwangversuch.

5. Zusammenfassung

Von 3 untersuchten Termitenarten reagierten *Heterotermes indicola* (Wasmann) und *Coptotermes amanii* (Sjöstedt) auf Kulturfiltrat verschiedener Schimmelpilze recht übereinstimmend. *Aspergillus flavus* IMI 89 717, *A. niger* ATCC 6275 und *Myrothecium verrucaria* OECD 6 schädigten diese beiden Arten, dagegen nicht die Trockenholztermite *Kalotermes flavicollis* (Fabr.). Durch Fraktionierung des Kulturfiltrates ließ sich zeigen, daß die Pilze ein bis drei Wirkungsmaxima aufweisen, also z. T. mehr als ein Toxin bilden, das Termiten schädigt. Das Maximum der Toxinbildung liegt innerhalb der ersten 3 Wochen des Pilzwachstums. Ältere Kulturen waren kaum noch giftig. Der Beginn der toxischen Wirkung wurde für *A. niger* auf festem Nährboden mit etwa 36—38 Stunden bestimmt. Für den benutzten Stamm von *A. niger* scheint das Ausmaß der Sporulation einen Einfluß auf die Toxinbildung zu haben. Offensichtlich entstehen die für Termiten toxischen Stoffe im Zusammenhang mit der Sporenbildung.

Summary

On the noxious effects of some mould fungi on termites

Out of 3 termite species investigated *Heterotermes indicola* (Wasmann) and *Coptotermes amanii* (Sjöstedt) reacted relatively identically on the culture

filtrates of different mould fungi. Aspergillus flavus IMI 89 717, *A. niger* ATCC 6275, and *Myrothecium verrucaria* OECD 6 were harmful to these 2 species, but not so to the drywood termite *Kalotermes flavicollis* (Fabr.). The fractional analysis of the culture filtrate revealed that the fungi have one to three effective maxima, i. e. they form partly more than one toxin which is harmful to termites. The highest amount of toxins is formed within the first 3 weeks of fungal growth. Older cultures were hardly toxic. With *A. niger* cultured on a solid medium the toxic effects began to act after about 36 to 38 hours. In the case of the strain of *A. niger* used the extent of sporulation seemed to influence the formation of toxins. The substances which are toxic to termites are obviously formed in connection with the process of sporulation.

Résumé

Effets néfastes de certaines moisissures sur les termites

Parmi 3 espèces de termites étudiés, *Heterotermes indicola* (Wasmann) et *Coptotermes amanii* (Sjöstedt) réagissaient d'une manière relativement identique sur les filtrats de culture de diverses moisissures. *Aspergillus flavus* IMI 89 717, *A. niger* ATCC 6275 et *Mryothecium verrucaria* OECD 6 étaient néfastes à ces 2 espèces mais pas au termite du bois sec *Kalotermes flavicollis* (Fabr.). L'analyse fractionée des filtrats de culture a montré que les champignons ont 1 à 3 maxima actifs c'est à dire qu'ils secrètent plus d'une toxine nuisible aux termites. La quantité la plus élevée de toxines se forme pendant les 3 premières semaines de la croissance fongique. Des cultures plus âgées n'étaient plus guère toxiques. Avec l'*A. niger* cultivé sur milieu solide, les effets toxiques ont commencé à se manifester après environ 36 à 38 heures. Dans le cas de la souche d'*A. niger* utilisée, le degré de sporulation semblait influencer la formation des toxines. Les substances toxiques aux termites se forment manifestement en corrélation avec le processus de sporulation.

Resumen

Acerca de los efectos dañinos de algunos hongos mohosos sobre los termites

De las tres especies de termites investigadas, *Heterotermes indicola* (Wasmann) y *Coptotermes amanii* (Sjöstedt) reaccionan relativamente en forma idéntica sobre los filtrados de cultivos de diferentes hongos mohosos. *Aspergillus flavus* IMI 89 717, *A. niger* ATCC 6275, *Myrothecium verrucaria* OECD 6 fueron dañinos a estas dos especies, por el contrario no lo fueron para el termite de madera seca *Kalotermes flavicollis* (Fabr.). El análisis fraccional de los filtrados de cultivos muestran que los hongos tienen una efectividad máxima de 1 a 3, esto es, que ellos forman parcialmente mas de una toxina que perjudica los termites. La máxima cantidad de toxinas se forman durante las tres primeras semanas del crecimiento fungoso. Los cultivos viejos fueron muy poco tóxicos. Los efectos tóxicos con cultivos de *A. niger* sobre un medio sólido comenzaron a actuar después de 36 a 38 horas. La extensión de las esporulaciones de la sepa de *A. niger* utilizada, mostró influencia en la formación de toxinas. Las sustancias que son tóxicas a los termites se forman obviamente en conección con el proceso de esporulación.

Schrifttum

BECKER, G.: Zerstörung des Holzes durch Tiere. In: MAHLKE-TROSCHEL-LIESE, Handbuch der Holzkonservierung. 3. Aufl. Berlin, 1950, 111—165.

BECKER, G.: Beiräge zur Prüfung der natürlichen Dauerhaftigkeit von Holz gegen Termiten. Holz als Roh- u. Werkstoff *19* (1961), 278—290.

BECKER, G.: Allgemeines über die Laboratoriumsprüfung der Beständigkeit von Werkstoffen und der Wirksamkeit von Schutzmitteln gegen Termiten. Materialprüf. *4* (1962), 215—221.

BECKER, G.: Über Haltung von Termiten im Laboratorium. Z. angew. Zool. *52* (1965 a), 385—398.

BECKER, G.: Versuche über den Einfluß von Braunfäulepilzen auf Wahl und Ausnutzung der Holznahrung durch Termiten. Material. u. Organismen *1* (1965 b), 95—156.

BECKER, G.: Über Beziehungen zwischen Tieren und Mikroorganismen im Holz. Material u. Organismen, Beih. 1 „Holz und Organismen", 1966 a, 481—496.

BECKER, G.: Einige Beobachtungen und Versuchsergebnisse an Zuchten von *Coptotermes*-Arten (Isopt.) im Laboratorium. Z. angew. Zool. *53* (1966 b), 463—479.

BECKER, G. und KERNER-GANG, W.: Schädigung und Förderung von Termiten durch Schimmelpilze. Z. angew. Ent. *53* (1964), 429—448.

FRANK, H. K.: Diffusion of aflatoxins in foods. J. Food Sci. *33* (1968), 98—100.

GRAHAM, K.: Fungal-insect mutualism in trees and timber. Ann. Rev. Entomol. *12* (1967), 105—126.

LENZ, M.: Zur Wirkung von Schimmelpilzen auf verschiedene Tierarten. Z. angew. Zool. *55* (1968), 295—374; 385—425.

LUND, A. E.: Are fungi beneficial to the termite diet? Termites and their attack on sound wood. Pest Control *28* (1960), 26—28.

MÜLLER-KÖGLER, E.: Pilzkrankheiten bei Insekten — Anwendung zur biologischen Schädlingsbekämpfung und Grundlagen der Insektenmykologie. Berlin, Hamburg, 1965.

SCHAEFFER, P.: Sporulation and the production of antibiotics, exoenzymes, and exotoxins. Bact. Rev. *33* (1969), 48—71.

Anschrift des Verfassers:

Dr. MICHAEL LENZ

Bundesanstalt für Materialprüfung
Berlin 45 (Dahlem)
Unter den Eichen 87

Contribution à l'étude de l'efficacité de la protection des bois traités par trempage long dans des solutions minérales

Par M. Fougerousse, J. Guillemain, C. Jacquiot
D. Lapetite, M. Roméis, MM. Serment

Centre Technique du Bois, Paris, et Centre Technique
Forestier Tropical, Nogent-sur-Marne (France)

Sommaire

1. Introduction
2. Conditions du débit et du traitement
3. Recherche qualitative de la profondeur de pénétration des produits
   3.1 Réactifs — 3.2 Résultats
4. Essais mycologiques sur Basidiomycètes
5. Essais entomologiques sur larves d'*Hylotrupes bajulus*
   5.1 Essai sur larves néonates d'*Hylotrupes bajulus* — 5.2 Essai de résistance au franchissement par des larves âgées de bois imprégnés par trempage avec différentes solutions de sels minéraux — 5.3 Observations
6. Essais de résistance aux attaques de *Reticulitermes santonensis*
   6.1 Mode opératoire — 6.2 Résultats
7. Dosages chimiques
   7.1 Méthodes de dosages utilisées — 7.2 Mode de prélèvement des échantillons et résultats analytiques — 7.3 Commentaires
8. Conclusions générales
9. Résumé

1. Introduction

Les essais de laboratoire auxquels sont soumis les produits de préservation en vue de l'homologation portent, le plus souvent, sur des éprouvettes imprégnées dans toute leur masse avec le produit à essayer. C'est, en particulier, le cas des essais d'action fongistatique tels qu'ils sont prévus par la Norme allemande DIN 52.176 et les Normes françaises X-41.502 et X-41.512.

On peut se demander s'il est légitime d'appliquer par une méthode de traitement de surface (trempage, aspersion en tunnel) des produits reconnus comme efficaces à la suite d'essais où ils étaient appliqués par imprégnation. Plus précisément, on peut se demander si le traite-

УДК 632.952

Фунгицидность жирноароматических кислот и их производных

Ч. Ш. КАДЫРОВ, С. В. ЛЕВУШКИНА, Г. Г. ГАЛУСТЬЯН

(Институт химии растительных веществ АН УзССР)

А. А. СУБХАНКУЛОВ, К. М. ЗАЙНУТДИНОВА, Р. Л. БИРЮКОВА

(Среднеазиатский институт фитопатологии)

Ранее сообщалось *, что некоторые синтетические фитогормоны повышают устойчивость растений к грибковым заболеваниям, причем у некоторых из них обнаруживаются фунгицидные свойства. В связи с этим нами были синтезированы и испытаны в качестве фунгицидов алифатические и жирноароматические кислоты и их производные.

Для испытаний использовали изолят гриба *Piricularia oryzae* Br. et. Cav., выделенный из растений риса в Краснодарском крае и выращенный на морковном агаре в пробирках, содержавшихся в неосвещенном термостате при 26—28°C. Все соединения испытывали в концентрации 50 *мг/л* и вводили в 2%-ный голодный агар в виде водной суспензии или эмульсии.

Полностью (на 100%) ингибировали развитие гриба *о*-толилмасляная, *н*-ксенилмасляная, толилундециловая, *о*-ксилилундециловая, *м*-ксилилундециловая и мезитилундециловая кислоты, а также *н*-пропилбутиролактон, бензилфуранкарбоновый эфир и бензоилксилол.

Результаты испытаний показали, что α-, β- и γ-арилмасляные кислоты слабо ингибируют развитие гриба. Введение алкил- гидрокси-, метокси- и фенокси-заменителей незначительно повышает активность препаратов. Только фенильный радикал C_6H_5 в *n*-положении к бензольному ядру резко (до 100%) усиливает ингибирующее действие соединений.

Активность препаратов падает до 0 при замене фенильного кольца на аценафтильный, нафталиновый и бензимидазолоновый остатки. Если карбоксильная группа в масляных кислотах заменена на нитрильную, то в ряде случаев повышается физиологическая активность препарата. При переходе от масляного остатка к пропионовому активность резко снижается. Снижает активность соединения и замена карбонильной группы α-метильным радикалом. Ароматические производные гептановой кислоты имеют несколько большую активность, чем подобные им производные масляной кислоты. Резкий скачок происходит при переходе от производных масляной кислоты к производным ундециловой кислоты.

Из всех испытанных в качестве фунгицидов замещенных 3-арилалкилкарбоновых кислот наибольший интерес представляют производные арилундециловых кислот.

Поступила в редакцию 21/VI 1968 г.

* Ч. Ш. Кадыров, Е. В. Бичеров, авт. свид. 172155, 1965 г.

УДК 632.95 : 661.718.1

Новые пестициды—смешанные амиды О-алкил-О-нитрокрезилтиофосфорной кислоты

Я. А. МАНДЕЛЬБАУМ, Л. И. ЛЕЩИНСКАЯ, Т. М. ПИВОВАРОВА

(ВНИИ химических средств защиты растений)

За последние годы внимание исследователей направлено на изыскание новых фосфорорганических пестицидов, обладающих сравнительно широким диапазоном действия и относительно низкой токсичностью для теплокровных животных. Из ряда препаратов, отвечающих этим требованиям, можно назвать О,О-диметил-О-3-метил-4 - нитрофенилтиофосфат (метатион [1], сумитион [2], фолитион [3]), а также метилнитрофос [4], представляющий собой смесь упомянутого соединения с О,О-диметил-О-3-метил-6-нитрофенилтиофосфатом, обладающим синергетическими свойствами.

Учитывая, что среди фосфорорганических соединений, содержащих в молекуле нитро-
крезолы ...
лотокси ...
синтезир ...
амидов ...
кислоты ...

KADŶROV, CH. SH., LEVUSHKINA, S.V., GALUST'YAN, G.G.
SUBKHANKULOV, A.A., ZAINUTDINOVA, K.M. & BIRYUKOVA, R.L.

1969

Fungitsidnost' zhirnoaromaticheskikh kislot i ikh proizvodnykh.

[Fungicidal activity of lipo-aromatic acids and their derivatives.]

- Khimiya sel' Khoz., 7 (9): 40.

[Inst. Chem. Pl. Substances, Uzbek Acad. Sci.]

Microfilm No. 15628

4. ...

5. ... н-C_3H_7NH ...

6. $(CH_3O)(\text{изо-}C_3H_7NH)P(S)OC_6H_3(CH_3)NO_2$-3,4

7. $(CH_3O)(\text{изо-}C_3H_7NH)P(S)OC_6H_3(CH_3)NO_2$-3,6

8. $(CH_3O)(\text{н-}C_4H_9NH)P(S)OC_6H_3(CH_3)NO_2$-3,4

9. $(CH_3O)(\text{н-}C_4H_9NH)P(S)OC_6H_3(CH_3)NO_2$-3,6

10. $(C_2H_5O)(CH_3NH)P(S)OC_6H_3(CH_3)NO_2$-3,4

11. $(C_2H_5O)(CH_3NH)P(S)OC_6H_3(CH_3)NO_2$-3,6

12. $(C_2H_5O)(C_2H_5NH)P(S)OC_6H_3(CH_3)NO_2$-3,4

13. $(C_2H_5O)(C_2H_5NH)P(S)OC_6H_3(CH_3)NO_2$-3,6

14. $(C_2H_5O)(\text{изо-}C_3H_7NH)P(S)OC_6H_3(CH_3)NO_2$-3,4

... -3,6

... 3,4

... 4

... 4

... 3,4

... 3,4

21. $(\text{н-}C_4H_9O)(CH_3NH)P(S)OC_6H_3(CH_3)NO_2$-3,4

22. $(\text{н-}C_4H_9O)(C_2H_5NH)P(S)OC_6H_3(CH_3)NO_2$-3,4

23. $(CH_3O)((CH_3)_2N)P(S)OC_6H_3(CH_3)NO_2$-3,4

24. $(CH_3O)((C_2H_5)_2N)P(S)OC_6H_3(CH_3)NO_2$-3,4

ИЗВЕСТИЯ АКАДЕМИИ НАУК СССР

№ 5—1969 Серия биологическая 656—659

УДК 576.8

М. С. ШКЛЯР, Э. О. САРДАРЯН

ИСПОЛЬЗОВАНИЕ БАКТЕРИЙ-АНТАГОНИСТОВ ДЛЯ БОРЬБЫ С ВОЗБУДИТЕЛЕМ АЛЬТЕРНАРИОЗА КАПУСТЫ — ГРИБОМ ALTERNARIA SP.

Испытана возможность применения бактерий-антагонистов для борьбы с возбудителем альтернариоза капусты — грибом Alternaria. В качестве антагонистов использовали бактерию рода Pseudomonas штамм 51 и производственный штамм Azotobacter chroococcum 56, широкий антифунгидный спектр которых был установлен лабораторными опытами.

Двухлетние опыты в парниках пригородных овощеводческих совхозов г. Ленинграда («Выборгский» и «Красный Октябрь») показали, что инокуляция не зараженных и зараженных грибом Alternaria семян капусты сорта Слава микробами-антагонистами оказала оздоровляющее действие на семена и на развитие растений. Особенно заметный эффект был получен при применении Az. chroococcum 56.

Вопрос об использовании микробов-антагонистов и антибиотиков для борьбы с болезнями сельскохозяйственн
леднее время внимание исследователей.
фактический материал, свидетельствующ
вания как живых культур микробов-ант
синтеза в виде антибиотиков. Можно на
(гризеофильвин, актидион, бластицидин
стен), прошедших успешное испытание в
не и за рубежом.

Однако у нас антибиотики еще не нац
ния в растениеводстве вследствие разлиı
ность, фитотоксичность, неустойчивость,
этим актуальной задачей являются поис
микробов-антагонистов и антибиотиков.

Целью нашей работы явилось испыта
бактерий-антагонистов для борьбы с во
капусты — грибом Alternaria.

Гриб Alternaria является распростра
ний многих сельскохозяйственных расте
мени еще нет достаточно действенных ме

Заболевание капусты альтернариозо
признаков (появление черных пятен на различных частях растений) в значительном снижении всхожести семян и в ослаблении роста капустной рассады.

SHKLYAR, M.S. & SARDARYAN,

Ispol'zovanie bakterii-ant
vozbuditelem al'ternarioza
sp. [Use of bacteria an
Cabbage.]

Izv. Akad. Nauk SSSR, Ser.

[4 tables. Engl. summ.

Microfilm no. 15

Из семян естественно пораженной альтернариозом капусты нами был выделен гриб Alternaria sp. оказавшийся достаточно вирулентным при искусственном заражении семян капусты сорт Слава. Заболеваемость при этом достигла 60% и более.

В качестве антагониста к испытанному на вирулентность грибу мы использовали Pseudomonas sp. штамм 51, выделенный нами из прикорневой зоны акации. Указанная бактерия, обладающая широким антигрибным спектром действия, при испытании в течение нескольких лет

в районах хлопкосеяния для борьбы с вилтом хлопчатника дала положительные результаты (Худяков и др. 1965; Пантелеев, Шкляр, 1966; Шкляр, Мансурова, 1967).

Вторым антагонистом служил Azotobacter chroococcum 56.

Обширная литература, посвященная всестороннему изучению азотобактера, объясняет положительное влияние этого микроорганизма на растение его способностью фиксировать атмосферный азот и продуцировать физиологически активные вещества (гетероауксин, витамины, гиббереллины).

В последние годы появились интересные исследования Е. Н. Мишустина с соавторами, показывающие, что азотобактер является антагонистом фитопатогенных грибов, в частности, Alternaria, что, возможно, является основной причиной его благоприятного влияния на растения. Это положение подтверждено авторами в опытах с искусственной инокуляцией яровой пшеницы и кукурузы грибом Alternaria и культурой азотобактера (Мишустин и др., 1963а, б; Марченко, 1963; Мишустин, Марьенко, 1965; Мишустин, Шильникова, 1968).

Таблица 1

Фунгистатическое действие различных видов и штаммов азотобактера к грибу Alternaria

Культуры азотобактера		Радиус зоны отсутствия роста гриба, *мм*
Az. chroococcum шт.	H_3	Нет зоны
То же	H_2	» »
» »	ОМ	11,5
» »	$Э_{12}$	Нет зоны
» »	$Э_{11}$	» »
» »	$Э_{10}$	2,5
» »	$Э_3$	Нет зоны
» »	$Э_1$	» »
» »	53	5,0
» »	56	26,5
» »	427	5,2
» »	16	2,5
» »	7	3,0
» »	33	17,5
Ar. beijerinckii		7,5
Az. niger		1,2
Az. indicum		Нет зоны
Ar. agile		6,2
Ar. vinelandii		1,2

О фунгистатическом действии азотобактера на возбудителей вил-

...RYAN, E.O. 1969

...ĭ-antagonistov dlya bor'bȳ s
...rioza Kapustȳ-gribom Alternaria
...ia antagonists against A. sp. on

... Ser. Biol., 1969 (5):656-659.
...umm.]

...5629

...ра нами было изучено несколько ви-
...ученных из музея живых культур и
...рений ВНИИСХМ. Антибиотическую
...и методом агаровых блоков на газо-
...ыращенного на сусло-агаре (табл. 1).
...пытанные виды азотобактера облада-

...ьшей антибиотической активностью к
...оососсum штамм 56, который и был

...ое испытание фунгистатического дей-
...гонистов — Pseudomonas штамм 51 и
...ию к 24 фитопатогенным грибам, ме-

...ктер 56 оказался антагонистом к 17, а
Pseudomonas 51 ... зволно взятых грибов.

С двумя отобранными антагонистами нами в течение двух лет (1967—1968 гг.) проводились опыты в парниках пригородных овощеводческих совхозов Ленинграда «Выборгский» и «Красный Октябрь». Опыты были поставлены в четырехкратной повторности. Площадь делянки каждой повторности 1,5 $м^2$. Продолжительность опытов 2 месяца.

Семена капусты сорт Слава инокулировали водной суспензией трехсуточной культуры гриба Alternaria sp. Опыты были поставлены по следующей схеме: 1) инокуляция семян грибом Alternaria sp.; 2) инокуляция грибом Alternaria sp. одновременно с культурой Pseudomonas штамм 51; 3) инокуляция грибом Alternaria sp. и суспензией азотобакте-

ИЗВЕСТИЯ АКАДЕМИИ НАУК СССР
№ 5—1969 Серия биологическая 656—659

УДК 576.8

М. С. ШКЛЯР, Э. О. САРДАРЯН

ИСПОЛЬЗОВАНИЕ БАКТЕРИЙ-АНТАГОНИСТОВ ДЛЯ БОРЬБЫ С ВОЗБУДИТЕЛЕМ АЛЬТЕРНАРИОЗА КАПУСТЫ — ГРИБОМ ALTERNARIA SP.

Испытана возможность применения бактерий-антагонистов для борьбы с возбудителем альтернариоза капусты — грибом Alternaria. В качестве антагонистов использовали бактерию рода Pseudomonas штамм 51 и производственный штамм Azotobacter chroococcum 56, широкий антифунгидный спектр которых был установлен лабораторными опытами.

Двухлетние опыты в парниках пригородных овощеводческих совхозов г. Ленинграда («Выборгский» и «Красный Октябрь») показали, что инокуляция не зараженных и зараженных грибом Alternaria семян капусты сорта Слава микробами-антагонистами оказала оздоровляющее действие на семена и на развитие растений. Особенно заметный эффект был получен при применении Az. chroococcum 56.

Вопрос об использовании микробов-антагонистов и антибиотиков для борьбы с болезнями сельскохозяйственных растений привлекает в последнее время внимание исследователей. Сейчас уже накопился большой фактический материал, свидетельствующий о перспективности использования как живых культур микробов-антагонистов, так и продуктов их синтеза в виде антибиотиков. Можно назвать целый ряд антибиотиков (гризеофильвин, актидион, бластицидин, трихотецин, агромицин, агристен), прошедших успешное испытание в растениеводстве в нашей стране и за рубежом.

Однако у нас антибиотики еще не нашли производственного применения в растениеводстве вследствие различных причин (слабая эффективность, фитотоксичность, неустойчивость, высокая стоимость). В связи с этим актуальной задачей являются поиски и испытание новых активных микробов-антагонистов и антибиотиков.

Целью нашей работы явилось испытание возможности использования бактерий-антагонистов для борьбы с возбудителем черной пятнистости капусты — грибом Alternaria.

Гриб Alternaria является распространенным возбудителем заболеваний многих сельскохозяйственных растений, однако до настоящего времени еще нет достаточно действенных мер борьбы с альтернариозами.

Заболевание капусты альтернариозом выражается помимо внешних признаков (появление черных пятен на различных частях растений) в значительном снижении всхожести семян и в ослаблении роста капустной рассады.

Из семян естественно пораженной альтернариозом капусты нами был выделен гриб Alternaria sp. оказавшийся достаточно вирулентным при искусственном заражении семян капусты сорт Слава. Заболеваемость при этом достигла 60% и более.

В качестве антагониста к испытанному на вирулентность грибу мы использовали Pseudomonas sp. штамм 51, выделенный нами из прикорневой зоны акации. Указанная бактерия, обладающая широким антигрибным спектром действия, при испытании в течение нескольких лет

в районах хлопкосеяния для борьбы с вилтом хлопчатника дала положительные результаты (Худяков и др. 1965; Пантелеев, Шкляр, 1966; Шкляр, Мансурова, 1967).

Вторым антагонистом служил Azotobacter chroococcum 56.

Обширная литература, посвященная всестороннему изучению азотобактера, объясняет положительное влияние этого микроорганизма на растение его способностью фиксировать атмосферный азот и продуцировать физиологически активные вещества (гетероауксин, витамины, гиббереллины).

Таблица 1

Фунгистатическое действие различных видов и штаммов азотобактера к грибу Alternaria

Культуры азотобактера	Радиус зоны отсутствия роста гриба, *мм*
Az. chroococcum шт. H_3	Нет зоны
То же H_2	» »
» » ОМ	11,5
» » Э$_{12}$	Нет зоны
» » Э$_{11}$	» »
» » Э$_{10}$	2,5
» » Э$_{3}$	Нет зоны
» » Э$_{1}$	» »
» » 53	5,0
» » 56	26,5
» » 427	5,2
» » 16	2,5
» » 7	3,0
» » 33	17,5
Ar. beijerinckii	7,5
Az. niger	1,2
Az. indicum	Нет зоны
Ar. agile	6,2
Ar. vinelandii	1,2

В последние годы появились интересные исследования Е. Н. Мишустина с соавторами, показывающие, что азотобактер является антагонистом фитопатогенных грибов, в частности, Alternaria, что, возможно, является основной причиной его благоприятного влияния на растения. Это положение подтверждено авторами в опытах с искусственной инокуляцией яровой пшеницы и кукурузы грибом Alternaria и культурой азотобактера (Мишустин и др., 1963а, б; Марченко, 1963; Мишустин, Марьенко, 1965; Мишустин, Шильникова, 1968).

О фунгистатическом действии азотобактера на возбудителей вилта хлопчатника — грибов Verticillium dahliae Kleb и Fusarium vasinfectum, говорят также данные А. А. Марго (1967). С целью отбора наиболее активных культур азотобактера нами было изучено несколько видов и штаммов азотобактера, полученных из музея живых культур и лаборатории бактериальных удобрений ВНИИСХМ. Антибиотическую активность азотобактера определяли методом агаровых блоков на газонах, засеянных грибом Alternaria, выращенного на сусло-агаре (табл. 1). За исключением Az. indicum все испытанные виды азотобактера обладали антибиотической активностью.

Как видно из табл. 1, наибольшей антибиотической активностью к грибу Alternaria обладает Az. chroococcum штамм 56, который и был использован в дальнейшей работе.

Мы также провели сравнительное испытание фунгистатического действия двух отобранных нами антагонистов — Pseudomonas штамм 51 и азотобактер штамм 56 по отношению к 24 фитопатогенным грибам, методом агаровых блоков (табл. 2).

Из табл. 2 видно, что азотобактер 56 оказался антагонистом к 17, а Pseudomonas 51 — к 20 из 24 произвольно взятых грибов.

С двумя отобранными антагонистами нами в течение двух лет (1967—1968 гг.) проводились опыты в парниках пригородных овощеводческих совхозов Ленинграда «Выборгский» и «Красный Октябрь». Опыты были поставлены в четырехкратной повторности. Площадь делянки каждой повторности 1,5 $м^2$. Продолжительность опытов 2 месяца.

Семена капусты сорт Слава инокулировали водной суспензией трехсуточной культуры гриба Alternaria sp. Опыты были поставлены по следующей схеме: 1) инокуляция семян грибом Alternaria sp.; 2) инокуляция грибом Alternaria sp. одновременно с культурой Pseudomonas штамм 51; 3) инокуляция грибом Alternaria sp. и суспензией азотобакте-

ра 56; 4) инокуляция грибом Alternaria sp. и смесью суспензий Pseudomonas 51 и Azotobacter 56; 5) семена, замоченные в воде. В качестве контролей служили 1-й и 5-й варианты.

Бактерии Pseudomonas вносили в виде семисуточной культуры, выросшей в жидком сусле и разбавленной в 1000 раз. Азотобактер вносили в виде пятисуточной культуры, смытой с агаризованной среды Эшби. Водные суспензии бактерий-антагонистов готовили с таким расчетом, чтобы в 1 *мл* содержалось 5—10 млн. клеток.

Таблица 2

Антагонистические свойства бактерий рода Pseudomonas штамм 51 и азотобактера штамм 56 (радиус зон отсутствия роста тест объектов, *мм*)

Тест объекты	Азотобактер штамм 56	Pseudomonas штамм 51
Penicillium glaucum	14,0	9,0
Verticillium dahliae	13,0	8,5
Botrytis cinerea	14,0	18,5
Aspergillus niger	Нет	5,0
Fusarium vasinfectum	2,5	1,5
Fusarium oxysporum	Нет	17,5
Fusarium avenaceum	1,5	18,5
Alternaria sp.	26,5	19,5
Ascochyta pisi	19,0	28,5
Mucor mucedo	Нет	Нет
Rhizoctonia Aderholdii	21,5	30,5
Trichoderma lignorum	2,2	3,2
Selerotinia bataticola	Нет	23,5
Sphaeropsis malorum	19,0	21,5
Helminthosporium sativum	Нет	19,5
Virosporia oryzaeceae	Нет	26,5
Piptoporus betulinus	32,5	25,5
Libertella betulina	32,5	28,5
Discula pinicola	21,0	22,5
Peniophora gigantea	31,0	25,0
Stereum purpureum	29,0	Нет
Coriorus hirsutus	30,0	25,0
Cerrena inicolor	Нет	Нет
Fomes fomentarius	29,5	Нет

В 1968 г. опыт был поставлен с заражением и без заражения растений грибом Alternaria.

Данные опыта приведены в табл. 3 и 4.

Как видно из табл. 3 и 4, инокуляция семян грибом Alternaria привела к снижению всхожести семян капусты и к уменьшению веса проростков. Оздоровляющее действие бактерий-антагонистов особенно наглядно проявилось на зараженном грибом Alternaria фоне (средний вес проростков в контроле 89,7—69,5 *г*; в варианте с бактерией Pseudomonas 94,2—156,8 *г*; в варианте с азотобактером 112,0—212,2 *г*). Одновременная инокуляция двумя антагонистами не дала преимуществ по сравнению с их раздельным внесением.

Гораздо менее заметный, но все же положительный эффект от инокуляции семян культурами бактерий-антагонистов был также получен и в опыте без искусственного заражения грибом. В данном случае могло сказаться как стимулирующее действие бактерий-антагонистов, так и их фунгистатическое действие. Последнее предположение в известной мере подтверждается результатами микробиологических анализов корешков, показавших, что в вариантах, обработанных антагонистами, особенно

Таблица 3

Влияние бактерий-антагонистов на рост капустной рассады
(опыт 1967 г.)

Варианты опыта	Средняя длина растений, *см*	Число растений	Средний вес одного растения, *г*	Общий вес растений, *г*	Средний вес растений, *г*
Без обработки (контроль)	22,2±0,50	121	2,9	359,0	89,7±2,5
Обработка семян культурой Alternaria	17,4±0,62	70	2,6	182,6	45,6±1,9
То же+культура Pseudomonas	22,9±0,30	116	3,2	376,9	94,2±3,6
То же+культура азотобактер	22,0±0,64	109	4,1	448,1	112,0±6,6
То же+Pseudomonas + азотобактер	22,8±0,84	113	4,2	475,4	118,8±5,7

Таблица 4

Влияние бактерий-антагонистов на рост капустной рассады
(опыт 1968 г.)

Варианты опыта	Незараженные растения				Зараженные растения			
	средняя длина растений, *см*	число растений	средний вес одного растения, *г*	общий вес растений, *г*	средняя длина растений, *см*	число растений	средний вес одного растения, *г*	общий вес растений, *г*
Контроль	21,0±0,7	105	5,15	541,5	18,0±0,65	77	3,61	278,0
Обработка семян культурой Pseudomonas	26,8±0,65	127	5,12	650,4	24,2±1,0	125	5,0	627,2
Обработка семян культурой азотобактер	27,1±0,04	143	5,80	818,3	25,5±0,09	165	5,14	848,8
Обработка семян культурами Pseudomonas и азотобактера	27,9±0,3	164	5,12	840,2	25,6±0,5	156	4,62	721,3

азотобактером, общее количество грибов было меньшим, чем в контрольных вариантах.

Микробиологические анализы также показали, что азотобактер лучше приживается в прикорневой зоне капусты, чем бактерии рода Pseudomonas.

ЛИТЕРАТУРА

Марго А. А. 1967. Сб. Микробиол. процессы, их роль в повышении плодородия почв и эффект удобрений, 97.

Марьенко В. Г. 1963. Докл. ТСХА, вып. 84. 301.

Мишустин Е. Н., Наумова А. Н., Марьенко В. Г. 1963а. Почвенная и сельскохозяйственная микробиология, Ташкент, стр. 7.

Мишустин Е. Н., Наумова А. Н., Марьенко В. Г. 1963б. Изв. ТСХА № 4 (53), 42.

Мишустин Е. Н., Марьенко В. Г. 1965. Микробиология, **XXXIV**, вып. 5, 866.

Мишустин Е. Н., Шильникова В. К. 1968. Биологическая фиксация атмосферного азота. М., «Наука».

Пантелеев А. А., Шкляр М. С., 1966. Хлопководство, № 12, 28.

Худяков Я. П., Шкляр М. С., Савадеров Е. П. 1965. Прикл. биохим. и микробиол., **1**, 2, 186.

Шкляр М. С., Мансурова М. Л. Бюл. Всесоюзн. н.-и. ин-та сельскохозяйств. микробиологии, № 14, вып. 1, 57.

Всесоюзный научно-исследовательский институт сельскохозяйственной микробиологии

Статья поступила в редакцию 19.IX.1968

M. S. SHKLIAR, E. O. SARDARIAN

UTILIZATION OF THE BACTERIA-ANTAGONISTS AGAINST THE AGENT OF ALTERNARIOSIS OF CABBAGE THE FUNGUS ALTERNARIA Sp.

The All Union Research Institute of Agricultural Microbiology

It was found that the bacterium of the Pseudomonas genus strain 51, isolated from the root zone of acacia as well as the industrial Azotobacter chroococcum strain 56 display a broad antifungal activity spectrum in particular against the causative agent of cabbage alternariosis — the fungus Alternaria.

Inoculation of cabbage seeds artificially infected with the fungus Alternaria with the said antagonists has produced a healing effect, which resulted in an increase of seed fertility and weight of seedlings as well as in a decrease of the fungus infection of the roots.

An especially noticeable effect was obtained in experiments with Azotobacter which has shown a higher vitality in the cabbage root system in comparison to Pseudomonas.

Simultaneous inoculation of cabbage seed with two antagonists has not shown any advantage in contrast to independent inoculation.

increasing external and internal demand; and (5) a long harvesting period. Table. Photos.

Royal Tropical Institute, Amsterdam

634.3:631.531.1(81) 634.3:581.14(81)
634.3:631.531.2(81)

s2277. **Soares M., C., and Donadio, L. C. Efeito da posição da semente de citros no tipo de seedling produzido.** (Effect of the position of the citrus seed on the type of seedling produced). Solo, Centro Acad. "Luiz de Queiroz", Brasil 60, 2, p. 69-70 (1968).

Root formation as influenced by seed position was investigated in São Paulo, Brazil, by sowing citrus seed horizontally, with the embryo-end up, or with the embryo-end down. Five months after sowing, the seedlings were pulled and their roots classified as follows: well-formed, tortuous, and very tortuous or snarled. It was found that when the seeds were sown horizontally with the embryo-end down, the roots were well-formed. Seeds sown with the embryo-end up resulted mostly in tortuous and very tortuous roots. From authors' English summary. Tables. Photos. 3 refs.

Royal Tropical Institute, Amsterdam

GHAZALI, S.W. 1968

Les Maladies à virus des agrumes au Liban. [The virus diseases of Citrus in Lebanon.]

Econ. Rurale Libanaise, 27:18-28. [Trop. Abstr., 24 (10):S2283.]

Microfilm no. 15630

in citrus nurseries. Best results were obtained with diuron applied at a rate of 0.2 or 0.3 g/m². After an application of 0.4 g/m² young citrus seedlings were slightly damaged. Recommended rates of the other 2 products are: BV-201 at 2.0 ml/m², and BV-207 at 1.5 ml/m². None of these products caused seedling damage. Tables.

Royal Tropical Institute, Amsterdam

634.3:631.531.2(81) 634.3:632.51(81)
632.954(81)

s2280. **Moreira, C. S., and Donadio, L. C. Contrôle químico de ervas daninhas em viveiro de citros.** (Chemical control of noxious weeds in a citrus nursery). Rev. Agr., Brasil 43, 3-4, p. 125-9 (1968).

Experiments were conducted in São Paulo, Brazil, to study the effect of diuron, dalapon and BV-207 on noxious weeds in a citrus nursery. In this nursery Rangpur lime, Rough lemon and *Poncirus trifoliata* were used as

rootstocks. Best results were obtained with diuron. No significant differences were found between the 3 rates of application of this herbicide, viz. 3, 5 and 8 kg per ha. The citrus seedlings were not affected by the herbicides applied. None of the products was effective against *Oxalis* sp. and *Cyperus rotundus*. English summary. Tables.

Royal Tropical Institute, Amsterdam

634.3:631.83(729.87) 634.3:581.192:631.8(729.87)
634.3:631.824(729.87) 634.3:631.559(729.87)
634.3:658.562(729.87)

s2281. **Weir, C. C. Potassium and magnesium nutrition of citrus trees.** Trop. Agr., Trinidad 46, 2, p. 131-6 (1969).

A field trial with citrus cv. "Valencia orange" (on sour orange), conducted in Trinidad showed that K fertilizing largely controlled leaf composition, yield and fruit quality. K applied at 0.5 or 1 kg K_2O/tree per year significantly depressed Mg, Ca, N and Zn in the leaves, but increased leaf-K. The 1st level of K fertilizer increased the yield by 16 %, but the 2nd dose depressed it by some 30 %. Similarly, K dressings significantly increased juice acidity and fruit size, but reduced juice content. Mg applied at 0.45 and 0.9 kg MgO/tree per year did not affect the quality. The data indicated an antagonistic relationship of K and Mg, an inverse relation of leaf-Ca and N, and a possible synergistic effect of Mg dressings on N, Mn and Zn in the leaves, whereas leaf-Cu was depressed. The results suggest the desirability of a strict control of K in the soil. Tables. Graphs. 11 refs.

Royal Tropical Institute, Amsterdam

634.3:632.161:631.811(68) 634.3:631.83(68)

s2282. **Bredell, G. S. Potassium deficiency in citrus.** Farming S. Africa 44, 11, p. 22-3 (1969).

In the Rep. of S. Africa, K deficiency has been noticed in citrus trees. Symptoms of a low K content are: small dark green leaves, which have sharp tips pointing upwards ("dogs' ears"); tendency of fruit to be very small, although trees generally have a good yield; and often die-back of small branches, which afterwards results in sparse foliage, with yellow leaves having small gum spots on the underside. Recommended (split) applications of KCl are: for trees from 1 to 5 years old, normally no K application; valencia trees from 5 to 10 years old, 0.9 kg per tree per year, and navels, 0.45 kg per tree per year; and for valencia trees from 10 years onwards, 1.8 kg per tree per year, and navels, 0.9 kg per tree per year. Under brackish conditions K_2SO_4, at 1¼ times the rate of KCl, is preferred. On sandy soils higher rates of applications are advisable. Photos.

Royal Tropical Institute, Amsterdam

634.3:632.388(569.34) 632.931(569.34)
632.97(569.34)

s2283. **Ghazali, S. W. Les maladies à virus des agrumes au Liban.** (The virus diseases of citrus in Lebanon). Econ. Rurale Libanaise 27, p. 18-28 (1968).

The symptoms, damage, transmission and control methods of the virus diseases of citrus occurring in Lebanon are described. At least 4 virus diseases of citrus are present in Lebanon, viz. psorosis *(Citrivir psorosis)*, xyloporosis-cachexy, stubborn *(C. pertinaceae)* and hard pit; exocortis and "tristeza" *(C. viatoris)* have never been observed. Four forms of psorosis exist: "psorose ecailleuse" *(C. psorosis* var. *vulgare)*, concave gum *(C. psorosis* var. *concavum)*, blind pocket *(C. psorosis* var. *alveatum)*, and crinkly leaf *(C. psorosis* var. *rugosum)*. General control measures recommended are: (1) production of disease-free planting material, using grafting tests on indicator plants, and of nucellar clones; and (2) strict quarantine measures. Table. Photos (1 coloured).

Royal Tropical Institute, Amsterdam

634.774:632.78(81) 632.951(81)

s2292. **Nakano, O., et al. Ensaio de campo visando o contrôle da broca do abacaxi (Thecla basilides Geyer).** (Field test on the control of the pineapple borer). Solo, Centro Acad. "Luiz de Queiroz", Brazil 60, 2, p. 29-31 (1968).

In São Paulo, Brazil, a field test was carried out on the control of the pineapple borer *(Thecla basilides)*. Treatments were: (1) 2.5 % disyston granules at 4 g per plant, applied in the leaf axils, 30 days prior to the emission of the flower peduncle; (2) 2.5 % disyston granules at 4 g per plant, plus 7.5 % carbaryl, dusted every 15 days after the emission of the flower peduncle; and (3) 7.5 % carbaryl dusted every 15 days, but initiated 30 days before the emission of the flower peduncle. Dusting with carbaryl proved to be a very efficient treatment for control of larvae. Disyston gave also significant control, as compared with the check, but was inferior to carbaryl. From author's English summary. Table. 6 refs.

Royal Tropical Institute, Amsterdam

634.84:632.4(54)

s2293. **Patil, L. K., and Moniz, L. A new anthracnose disease of grape vine from India.** J. Univ. Poona, Sci. Technol. Sect. 36, p. 107-10 (1969).

In India, grape vines *(Vitis vinifera)* were seriously affected by an anthracnose disease characterized by the drying of foliage and the shrinkage of berries. *Botrysdiplodia palmarum,* a fungus known to infect unripe coconuts in Africa, was identified as the causal organism. The pycnidia and pycnidiospores of the fungus are described. Photo. 3 refs.

Royal Tropical Institute, Amsterdam

634.84(689.4) 634.84:631.52(689.4)
634.84:631.5(689.4)

s2294. **Hatchwell, R. M. de P. Grapevine cultivation in the Central Province.** Farming Zambia 4, 2, p. 15 (1969).

Preliminary trials with 25 cultivars of grapevine *(Vitis vinifera)* conducted in Zambia showed that the American cv. "Catawba" was the most promising in all respects. Some 18 months after planting of cuttings ripe bunches may be harvested for both table use or wine production. The table cv. "Green Muscat" produces some 9 kg fruit of bunches twice per year, with bunch weights averaging about 0.7 kg each. The cv. "Black Prince" lacks the fruit properties for table consumption. Seven other cultivars showed only mediocre results. Various cultivation conditions, among others, location, manuring, planting, disease and pest control are briefly mentioned. Observations showed that after pruning in May, instead of July, fruit bunches ripen in Dec. prior to availability of imported grapes. Photo.

Royal Tropical Institute, Amsterdam

Roots and tubers

633.491:631.67(569.34) 633.491:631.67:626.845(569.34)
633.63:631.67(569.34) 633.63:631.67:626.845(569.34)
635.25:631.67(569.34) 635.25:631.67:626.845(569.34)

s2295. **Sarref, S. G. Détermination de la dose d'irrigation de certaines plantes cultivées au Liban.** (Determination of amount of irrigation water of certain cultivated plants in Lebanon). Econ. Rurale Libanaise 28, p. 21-34 (1968).

For assessment of the most effective furrow and sprinkler irrigation methods, commonly practised by the farmers in Lebanon, field trials were conducted with potatoes, sugar-beets, and onions at 3 localities in 2 different agro-climatic regions. In the Bekaa region, it was found that for furrow irrigation of potatoes the most suitable method was to apply 400-500 m^3/ha at 7-day

intervals. In the case of sprinkling, a rate of 400 m³/ha at 8-day intervals was most suitable for potatoes, and a rate of 500 m³/ha at 9-day intervals for sugar-beets and onions. With furrow irrigation of potatoes in the northern Abde region, a rate of approx. 500 m³/ha applied every 9 days proved to be best. Tables.

Royal Tropical Institute, Amsterdam

See also Books, s2371.

633.493:382(4) 636.086.4(4)

s2296. **L'avvenire della manioca nella comunità Europea.** (The prospects of cassava in the European Common Market). Agri Forum 10, 2, p. 57-8 (1969).

The actual situation and future prospects of the import of cassava, and its use in compound animal feeds, in W. Europe are analysed. The situation of W. Germany is compared with those of the Netherlands and Belgium. The cassava imports of the 3 mentioned countries doubled to 800,000 tons from 1962 to 1967. Factors largely responsible for this are: (1) increased numbers of cattle, pigs and poultry, and increased demand for animal products; and (2) rapid development of an animal feed industry, and an increased use of compound feeds in which cereals are partly substituted by cheaper cassava.

1969

PATIL, L.K. & MONIZ, L.A.

A new anthracnose disease of Grapevine from India.

J. Univ. Poona, 36:107-110. [Trop. Abstr., 24 (10): S 2293]

Microfilm no. 1563

maximum 4 hrs, and enough salt, e.g. potassium phosphate, is added as a yeast nutrient. Weight loss during baking is only 10 to 11 %, against 13 % for bread made from wheat flour alone. Analysis conducted in Puerto Rico showed that the nutritive value of the new type of bread compares favourably to that of bread made from wheat flour alone. Table. Photos.

Royal Tropical Institute, Amsterdam

Rubber

633.912.11:631.116.6(595+911.11+911.14)
633.912.11:657.47(595+911.11+911.14)

s2299. **[Malaysia's rubber] — making the most of it.** Rubber Developments 22, 1, p. 20-4 (1969).

Changes in the world rubber price have a great influence on the family

Radochová & Málek, 1969b) as only four phenotypes were common for both, i.e. glycine⁻ (serine⁻); serine⁻; thiamine⁻ and purines⁻. Mutants requiring glycine or serine were most frequent. However, mutants of a related phenotype were not obtained as compared with nitrosoguanidine treatment (Koníčková-Radochová & Málek, 1969a). The mutants requiring lysine or purines were considerably less frequent and mutants requiring pyrimidines were not detected at all. Roughly 1/4 of induced auxotrophic strains could be used in further genetic studies due to a low frequency of spontaneous reversions. This quantity of mutants is rather high

shoul

stabil

is st

micro

showe

of au

treati

12%

worse in our model. The genetic instability is most clearly demonstrated in the mutants of the glycine⁻ (serine⁻) phenotype.

EMS exhibited a high mutagenic effect even on the other studied markers, i.e. induction of resistance to INH and resistance to STM. In *Escherichia coli* Verly *et al.* (1967) found that the frequency of STM-resistant mutants was high particularly in using higher EMS concentrations (0.16 M). However, a longer time was required for their phenotypic expression in order to reach a maximum frequency of induced mutants (as compared with other mutagens, e.g.

er

u-

ed

on

ve

vá

1969

BARÁTHOVÁ, H., BETINA, V. & NEMEC, P.

Morphological changes induced in fungi by antibiotics.

Folia microbiol., Praha, 14 (5):475-483. [5 tables.
Fac. chem. Techn., Bratislava.]

Microfilm no. 15832

Refer

Bautz, ... *alkyl* ... 46 : 1 ... nd ... 36,

Koníčk ... *genic* ... *phlei* ... as ... vi- ... on

Koníčk ... *genic* ... *phlei* ... at ... te.

Koníčk ... *tion of auxotrophic mutants in Mycobacterium phlei PA by ultraviolet radiation.* Fol. microbiol. 14 : 251, 1969b.

Krieg, D. R : *Ethylmethansulphonate-induced reversions of bacteriophage T4 rII mutants.* Genetics 48 : 561, 1963.

Lawley, P. D., Brookes, P.: *Acidic dissociation of 7 : 9-dialkylguanines and its possible relation to mutagenic properties of alkylating agents.* Nature 192 : 1081, 1961.

Lawley, P. D.: *Molecular mechanism of the cytotoxic action of difunctional alkylating agents and of resistance to this action.* Nature 206 : 480, 1965.

Lindegren, G., Hwang, J. L., Oshima, Y., Lindegren, C. C.: *Genetical mutants induced by ethylmethansulfonate in Saccharomyces.* Can. J. Genet. Cytol. 7 : 491, 1965.

Loprieno, N.: *Differential response of Schizosac-*

Nečásek, J.: *Mutagenic effect of ethyl methanesulfonate in Corynebacterium. The physiology of gene and mutation expression.* Proceedings of a Symposium held in Prague, 1965, Academia — Prague, 115, 1966.

Straus, B. S.: *Specificity of the mutagenic action of the alkylating agents.* Nature 191 : 730, 1961.

Tessman, I., Poddar, R. K., Kumar, S.: *Identification of the altered bases in mutated single-stranded DNA. I. In vitro mutagenesis by hydroxylamine, ethyl methanesulfonate and nitrous acid.* J. mol. Biol. 9 : 352, 1964.

Verly, W. G., Barbason, H., Dusart, J., Petitpas-Dewandre, A.: *A comparative study of the action of ethyl methane sulfonate and HNO_2 on the mutation to streptomycine resistance of Escherichia coli K 12.* Biochim. biophys. Acta 145 : 752, 1967.

Morphological Changes Induced in Fungi by Antibiotics

H. BARÁTHOVÁ, V. BETINA and P. NEMEC

Department of Microbiology and Biochemistry, Slovak Technical University, Faculty of Chemical Technology, Bratislava

Received July 5, 1968

ABSTRACT. In tests of 31 antibiotics, 29 inhibited growth of *Botrytis cinerea* and of these, 18 induced morphological changes. Terminal and lateral branching of the hyphae was induced by actinomycin D, aspergillic acid, citrinin, cyanein, cycloheximide, desertomycin and polyene antibiotics. Curling of the hyphae was induced by griseofulvin and narrowing of the hyphae by citrinin. Some antibiotics at different concentrations produced several types of morphological changes. For example, aspergillic acid, desertomycin and flavofungin also induced terminal bulging of the hyphae. Growth of the dimorphic fungus *Paecilomyces viridis* was inhibited by 24 antibiotics, nine of which induced morphological changes. Branching of the hyphae was induced by azalomycin F, citrinin, cyanein, desertomycin, patulin, rugulosin and trichothecin. Griseofulvin had a curling effect. Except for rugulosin, the above antibiotics, in higher concentrations, induced yeast-like growth of *Paecilomyces viridis*. Morphological changes were also induced by inhibitors of RNA and protein synthesis and by antibiotics injuring the cell membranes. Antibiotics with different mechanisms of action induced similar morphological changes.

Various authors have studied the effects of antibiotics on the morphology of fungi. Brian *et al.* (1946) observed curling of the hyphae of *Botrytis cinerea* after contact with griseofulvin. Klinkovski (1961) found that polyene antibiotics induced bulging funnel-like formations in *Fusarium culmorum.* Hütter *et al.* (1965) divided a number of antifungal antibiotics into three groups, according to the morphological changes they induced in *Botrytis cinerea*; griseofulvin and ascomycin had a curling effect, filipin had a bulging effect and scopamycins induced branching of the hyphae. Cyanein also induced several types of morphological changes in different fungi (Betina *et al.*, 1965, 1966). Factors inducing vacuolation, branching and other effects in fungi were described by Park and Robinson (1964) and Robinson and Park (1965, 1966).

When studying antifungal metabolites of fungi we frequently observed morphological changes in *Botrytis cinerea.* We therefore tested the effect of 31 antifungal antibiotics with different mechanisms of action on the morphology of *Botrytis cinerea* and the dimorphic fungus *Paecilomyces viridis* (Segretain *et al.*, 1964), with the aim of determining the association between the types of morphological changes induced and the mechanism of action of the antibiotics.

MATERIALS AND METHODS

Microorganisms: The strain *Botrytis cinerea* 4-22 from our collection and a strain of *Paecilomyces viridis* from the Mycothèque du Service de Mycologie, Institut Pasteur, Paris, were maintained on malt agar slants and the same medium was used for testing. The cultivation temperature was 25° C.

Antibiotics: For predetermining morphological effects we used 31 antifungal antibiotics: actinomycin D, amphotericin B, aspergillic acid, aurantiogliocladin-rubrogliocladin (1 : 1), azalomycin F, baccatin A (a mixture of enniatin A and B, 3 : 1; Hall, 1960), citrinin, cy-

anein, cycloheximide (actidione), cyclopaldic acid, dehydrofusaric acid, desertomycin, enniatin B, filipin, flavipin, flavofungin, frequentin, fusaric acid, gladiolic acid, gliotoxin, griseofulvin, kojic acid, 5-methoxy-*p*-toluquinone, mycophenolic acid, neohydroxyaspergillic acid, nystatin, patulin, rugulosin, terreic acid, trichothecin and viridin.

The effects of actinomycin D, aspergillic acid, citrinin, cyanein, cycloheximide, desertomycin, flavofungin and griseofulvin on the morphology of growing hyphae of the two test microorganisms and on the germination of their conidia were compared in detail.

The growth curves of the test microorganisms in the presence of these eight antibiotics were also studied. Standard antibiotic solutions in dimethylsulphoxide were added to sterile agar before plating, in a final concentration of 1% of the solvent.

Determination of morphological effect of antibiotics on growing hyphae: We used the paper disc technique (Hütter *et al.*, 1965) with the following modifications. Agar in Petri dishes 10 cm in diameter was inoculated in the centre of the dish with one loop of a standard *Botrytis cinerea* spore suspension. The plates were incubated for 48 hours at 25° C. The antibiotics, dissolved in suitable solvents, were applied to discs in amounts of 200, 20 and 2 μg/disc. After drying, the discs were placed on the agar 5 mm from the edge of the growing colonies. The inhibitory effect and morphological changes were examined after a further 24 hours' incubation at 25° C.

In some experiments with antibiotics we used another method. Blocks 8 mm in diameter were cut from the edge of a 72-hour *Botrytis cinerea* colony and were applied to Whatman No. 1 filter paper discs (⌀ 1 cm) placed in the centre of Petri dishes containing malt agar. After 24 hours' incubation at 25° C the hyphae had grown beyond the edge of the discs. The latter were then removed, together with the block, leaving a ring of hyphae of the same age, 3—4 mm in diameter, on the agar. Paper discs with antibiotic were applied to the outer edge of this ring and the plates were incubated 24 hours at 25° C. They were then stained with cotton blue and changes were observed under the microscope.

In tests with *Paecilomyces viridis* we used 96-hour colonies growing in the centre of Petri dishes. The character of growth and morphological changes were observed 24—96 hours after applying the discs with antibiotics.

Effect of antibiotics on conidia germination: Slide cultivation was used, according to the method of Segretain *et al.* (1963). A thin agar layer already containing antibiotic solution was applied to each slide. The final solvent concentration (1%) was the same both in the antibiotic agar and the control. The inoculum for microculture inoculation was prepared by the technique of El-Nakeeb *et al.* (1965).

Effect of antibiotics on growth of colonies: Antibiotic solutions in dimethylsulphoxide were added to agar in final concentrations of 100, 50, 10 and 5 μg/ml. The dimethylsulphoxide concentration was 1%, both in the experimental material and the control. For each concentration we used one Petri dish with three colonies. At intervals during incubation at 25°C we measured the diameters of the colonies in two directions perpendicular to each other. We observed the shape of the colonies and examined the morphology of the marginal hyphae under the microscope.

RESULTS

Effects of antibiotics on *Botrytis cinerea*

Effect on morphology of growing hyphae. Growth was inhibited to a greater or lesser degree by 29 of the 31 antibiotics tested; flavipin and rugulosin had no

Table 1. Morphological effects on growth of *Botrytis cinerea* observed after 24 hours' growth on malt agar at 25° C. (The figures in circles denote the effect after only 6 hours)

Antibiotics	Active conc., (μg/disc)	Morphological effects*							
		A			B	C	D	E	F
		A_1	A_2	A_3					
Actinomycin D	200	−	−	+	−	−	−	−	−
Aspergilic acid	200, 20	⊕	−	−	−	−	+	+	−
Aurantiogliocladin + rubrogliocladin (1 : 1)	200, 20	⊕	−	−	−	−	−	−	−
Azalomycin F	200, 20, 2	⊕	+	−	−	−	−	−	+
Baccatin A	200, 20	−	−	+	−	−	−	−	−
Citrinin	200	+	+	−	−	+	−	−	−
Cyanein	200, 20	+	−	−	−	−	+	−	−
Cycloheximide (actidione)	200	−	+	−	−	−	−	−	+
Desertomycin	200	⊕	−	−	−	−	−	⊕	+
Desertomycin	20	−	+	−	−	−	−	−	−
Enniatin B	200, 20	−	+	−	−	−	−	−	−
Filipin	200	−	−	+	−	−	−	−	−
Flavofungin	200	+	−	−	−	−	+	+	+
Flavofungin	20	−	+	−	−	−	+	−	−
Gliotoxin	200	−	−	+	−	−	−	−	−
Griseofulvin	200, 20, 2	−	−	+	⊕	−	−	−	−
Nystatin	200, 20, 2	+	−	−	−	−	−	−	+
Terreic acid	200	−	+	−	−	−	−	−	−
Trichothecin	200, 20	−	−	+	−	−	−	−	−
Viridin	200, 20	⊕	−	−	−	−	−	−	−

* A — branching: A_1 — terminal; A_2 — lateral; A_3 — irregular; B — curling; C — narrowing; D — broadening; E — bulging; F — emptying.

effect. Twenty antibiotics induced different types of morphological changes (Table 1).

For detailed study we selected eight antibiotics representing all types of induction of morphological changes. When choosing them we also took into account the mechanism of their action. They were tested in concentrations 100 and 10 μg/disc. Some of them, e.g. desertomycin and flavofungin, induced 2—3 types of morphological changes, according to the concentration.

The effect of griseofulvin was manifested after only 3 1/2 hours' contact. In both concentrations the well known curling effect was observed (Plate 1b).

Cyanein had the same effect as in earlier experiments (Betina *et al.*, 1966). In the higher concentration it induced terminal branching of the hyphae and partial broadening and shortening of the cells (Plate 1c).

After 3 1/2 hours' contact with aspergillic acid we observed terminal branching of the hyphae. In the presence of a concentration of 100 μg/disc, 3—4 short outgrowths were produced. After 22 hours' contact, primary to tertiary branching could be observed; the hyphae were 1 1/2 times broader and the cells were shorter. Empty vacuoles were formed at the tips of the hyphae (Plate 1e). In the presence of the lower aspergillic acid concentration the branches were longer and narrower (Plate 1d).

In addition to the narrowing effect of citrinin described by Robinson and Park (1966) we also found branching of the hyphae of *Botrytis cinerea*. The hyphae had branched ends and large numbers of short branches grew out perpendicularly

from the main filaments. They were about 50% narrower than in the control (Plate 1f).

The higher flavofungin concentration had a destructive effect on the hyphae. The cytoplasm became granulated, the hyphae, in places, were vacuolated, some

Table 2. Types of morphological effects of 6 antibiotics on *Botrytis cinerea*

Antibiotics	Types of effects* for concentrations of	
	10 μg/disc	100 μg/disc
Griseofulvin	A_3, B	A_3, B
Cyanein	—	A_1, D
Aspergillic acid	A_1, D	A_1, D, E
Citrinin	—	A_1, A_2, C
Flavofungin	A_2	A_1, F, D
Desertomycin	A_2	A_2, E, F

* For explanation see Table 1.

of the cells were broadened and the ends of the hyphae were branched (Plate 1h). The lower concentration induced the formation of short outgrowths perpendicular to the main filament (Plate 1g).

The types of the effects of the antibiotics illustrated in Plate 1 are given in Table 2.

Cycloheximide severely inhibited the growth of *Botrytis cinerea*. As with citrinin and desertomycin, an increase in the incidence of lateral hyphae was observed in the presence of the higher concentration. In 100 μg concentration, desertomycin induced broadening and terminal bulging of the hyphae; in both concentrations it caused granulation of the cytoplasm (Plate 1i).

Actinomycin D induced irregular branching combined with a pronounced inhibitory effect.

Effect of selected antibiotics on germination of conidia. In the germinating control, several straight, regularly branched hyphae about 20 cells long were found after 24 hours. Regularly growing colonies were formed within 48 hours and sporulation was observed after 48—72 hours.

In 100 μg/ml concentration, actinomycin D still completely inhibited germination of the conidia after 72 hours. In 10 μg/ml concentration it partly inhibited growth. Aspergillic acid in 100 μg/ml concentration completely inhibited germination. In 10 μg/ml concentration it inhibited growth and after some time induced morphological changes in the hyphae. After 72 hours the hyphae were bent and the granulated cytoplasm resembled a necklace. After 120 hours' contact with the antibiotic, chlamydospore-like formations appeared (Plate 2a).

Citrinin in 100 μg/ml concentration partly inhibited growth of the hyphae; the conidia germinated as in the control, the only difference being that the cells were shorter and the number of branches was greater. After 48—72 hours branching and narrowing of the hyphae was more pronounced and was the same as described above (Plate 2b).

The effect of 100 μg/ml cyanein during the first 24 hours was the same as that of citrinin. After 48 hours, growth of the hyphae in dense clusters was observed. The marginal hyphae had branched ends. Morphological changes were also observed in the presence of 10 μg/ml cyanein. Cycloheximide completely inhibited germination in 100 μg/ml concentration; in 10 μg/ml concentration it inhibited growth. In the presence of 50 μg/ml desertomycin, 2—3 cells grew in 24 hours and the hyphae were bent; the same situation was observed at 72 hours. A lower concentration (10 μg/ /ml) inhibited growth.

Flavofungin in 100 μg/ml concentration inhibited germination of the conidia. Signs of germination were observed after 24 hours; at 48 hours the conidial contents stainable by cotton blue had shrunk to the centre of the conidium which became prolonged and thinned out towards

the centre. After 120 hours, 2—3 irregular, bulging cellular formations appeared. After 24 hours' contact with a 10 μg/ml concentration the cells were shorter, broader and more frequently branched.

Griseofulvin was tested in 10 and 1 μg/ml concentrations. Both induced curling of the hyphae. Germination of the conidia was partly inhibited by the higher concentration. One to two curled hyphae grew during the first 24 hours, there was no further pronounced growth during the next 24 hours, but the cytoplasm was granulated. A large number of short hyphae grew in the presence of the lower concentration and later on growth in clusters was observed.

Table 3. ID_{50} values, minimal inhibitory concentrations (MIC) and minimal antibiotic concentrations inducing morphological changes in *Botrytis cinerea* on malt agar

Antibiotics	ID_{50} (μg/ml)	MIC (μg/ml)	Minimal conc. (μg/ml) inducing morph. changes
Actinomycin D	13	50	—
Aspergillic acid	15	50	10
Citrinin	120	>100	100
Cyanein	40	>100	50
Cycloheximide (actidione)	4	100	5
Desertomycin	2.5	50	5
Flavofungin	1.8	10	5
Griseofulvin	6	100	5

Inhibition of growth of colonies by selected antibiotics. The growth of colonies was measured in the presence of antibiotic concentrations of 100, 50, 10 and 5 μg/ml. We simultaneously studied the morphological effects, which were similar to those described above. Citrinin, desertomycin and cyloheximide (actidione) prolonged the lag phase from 24 to 44 hours. Table 3 gives the ID_{50} values, the minimal inhibitory concentrations and the minimal concentrations inducing morphological changes after 96 hours' cultivation.

Effects of antibiotics on *Paecilomyces viridis*

Effect on morphology of growing hyphae. Growth was inhibited by 24 of the 31 antibiotics tested; it was unaffected by amphotericin B, flavipin, gladiolic acid, kojic acid, 5-methoxy-*p*-toluquinone, neohydroxyaspergillic acid and terreic acid. Nine antibiotics which induced morphological changes in *Paecilomyces viridis* are given in Table 4, with details of the type of changes induced. Of the morphological changes observed in *Botrytis cinerea*, only types A and B occurred in *Paecilomyces viridis*; the curling effect of griseofulvin was different. The hyphae did not branch, but were only curled. Isaac and Milton (1967) observed a similar effect by griseofulvin in *Verticillium albo-atrum*, which is taxonomically close to *Paecilomyces viridis*. Several antibiotics induced yeast-like growth (Betina *et al.*, 1966).

Table 4. Morphological effects of antibiotics on *Paecilomyces viridis* after 96 hours' cultivation on malt agar at 25° C. (The figures in circles denote the effect after 24 hours)

Antibiotics	Concentration (μg/disc)	Morphological effects*		
		A	B	G
Azalomycin F	200, 20, 2	+	—	+
Citrinin	200, 20	+	—	+
Cyanein	200, 20, 2	⊕	—	+
Desertomycin	200, 20	⊕	—	+
Griseofulvin	200, 20, 2	—	⊕	—
Patulin	200, 20	+	—	+
Rugulosin	200	+	—	—
Trichothecin	200, 20	+	—	+
Viridin	200	—	—	+

* A — branching; B — curling; C — yeast-like form.

Actinomycin D, citrinin, cyanein, desertomycin and griseofulvin were chosen as inducers of all three types of morphological changes, for detailed study of their effects on *Paecilomyces viridis*. They were used in concentrations of 100 and 10 μg/disc.

As in *Botrytis cinerea*, the higher citrinin concentration induced lateral branching in 48 hours. After 72—96 hours, broadened formations composed of short, rounded cells were observed. Cyanein, in both concentrations, induced the branching described by Betina *et al.* (1966).

Desertomycin, after 24 hours, displayed a similar effect to that of cyanein. Brown pigment appeared on the under side of the colony, which puckered, and broadened hyphae with rounded cells were formed.

Effect on germination of conidia of Paecilomyces viridis. The 24-hour control showed 2—3 straight hyphae, branched in places, with a length of 10—15 cells. A colony grew by 48 hours and sporulation occurred by 72 hours.

Actinomycin D, citrinin and desertomycin did not affect germination, but inhibited growth. Cyanein, in both concentrations, severely inhibited growth and induced the formation of yeast-like cells: in the lower concentration it caused branching of the hyphae. Growth in the presence of the higher concentration was of the yeast-like type throughout the whole time of observation, Plate 3b). In the presence of the lower cyanein concentration the terminal cells were filamentous and not yeast-like.

In the higher concentration griseofulvin similarly to cyanein, induced yeast-like grown. In the lower concentration it caused curling.

Inhibition of growth of colonies. Growth and morphological changes in colonies on malt agar were observed for 12 days, using the selected antibiotics in concentrations of 100, 50, 10 and 5 μg/ml. Microscopic changes in the hyphae were similar to those described above. The morphological effect of actinomycin D was not manifested until after six days. All five antibiotics induced more or less similar changes in the shape of the colonies, which were puckered and grew upwards. The ID_{50} values, the minimal inhibitory concentration and the minimal concentrations inducing microscopic and macroscopic changes are given in Table 5.

DISCUSSION

When studying the morphological effects of antibiotics on *Botrytis cinerea* we found that the majority of them induced morphological changes in the hyphae. These antibiotics cannot be divided into distinct groups according

Table 5. ID_{50} values, minimal inhibitory concentrations (MIC) and minimal antibiotic concentrations inducing microscopic and macroscopic changes in *Paecilomyces viridis* after 12 days' growth on malt agar at 25°C

Antibiotics	ID_{50} (μg/ml)	MIC (μg/ml)	Minimum conc. (μg/ml) inducing morphological changes	
			microscopic	macroscopic
Actinomycin D	26	> 100	50	100
Citrinin	50	> 100	50	50
Cyanein	6	100	10	10
Desertomycin	6	100	5	5
Griseofulvin	10	100	5	10

to which type of morphological change they induce. In some cases the effect of the antibiotic varied with its concentration and the same antibiotic induced several types of changes (e.g. citrinin, desertomycin, flavofungin). The antibiotic concentrations needed for the induction of changes were several times lower than the minimum inhibitory concentrations (see Table 3). Morphological changes were observed only in hyphae directly in contact with the agar, none occured in the aerial mycelium.

The most frequent type of change was branching. This was induced by inhibitors of nucleic acid synthesis, such as actinomycin D and cycloheximide (Kerridge, 1958), and by inhibitors of protein-synthesis, such as cyanein (Drobnica & Betina, 1966; Betina & Montagnier, 1966). Polyene antibiotics (azalomycin F, flavofungin, nystatin), which injure the function of the cell membranes, also caused branching. A number of other antibiotics which induce morphological changes in *Botrytis cinerea*, also directly or indirectly impair the function of the membranes or the cell wall, as well as interfering in cellular metabolism. Pastan and Friedman (1968) found that actinomycin D inhibited chick fibroblast phospholipids and concluded that it might inhibit synthesis of the membranes. Cycloheximide induces giant cell formation by *Saccharomyces pastorianus*; these changes could be due to the effect of cycloheximide on synthesis of the material for the cell wall (Gundersen & Wadstein, 1962). Citrinin, cyanein and desertomycin release riboflavin from the mycelium of *Eremothecium ashbyi* and some pigments from plant and animal cells (Betina & Baráthová, 1968; Baráth *et al.*, in press; Betina *et al.*, 1969). Enniatin B and baccatin A are depsipeptide antibiotics which react with the cell membrane lipoproteins and affect K^+ ion transport in the mitochondria (Shemyakin, 1965; Pressman, 1965). Griseofulvin destroys the integrity of the cell wall and causes broadening (Blank *et al.*, 1960). In high concentration it causes enlargement and rupture of the cells (Aytoun, 1956; Brian *et al.*, 1949). Hessayon (1951) observed a similar phenomenon on using trichothecin.

Most of the antibiotics tested affect different metabolic processes and the morphological changes are probably not the outcome of their direct action. Antibiotics with different mechanisms of action induced similar morphological changes. We should look for the causes of morphological changes at a deeper level, in metabolism. The induction of morphological changes in growing hyphae could, however, be associated, either primarily or secondarily, with changes in membrane permeability and with impairment of cell wall synthesis. Thomas and Mullins (1967) attribute the induction of branching in male hyphae of Achlya to "softening" of the cell wall through the action of cellulase.

A significantly smaller number of the antibiotics tested induced morphological changes in the dimorphic fungus *Paecilomyces viridis*. In addition to curling and branching effects we observed the induction of yeast-like growth of this fungus.

Yeast-like forms of dimorphous fungi can be induced *in vitro* by high temperatures, by an increase in the partial CO_2 pressure in the atmosphere, by a raised concentration of —SH or —S—S— substances, by vitamins, etc. (Mariat, 1964; Gilardi, 1965). Yeast-like growth of *Mucor rouxii* can be induced by anaerobic cultivation in the presence of a high partial CO_2 pressure (Bartnicki-Garcia & Nickerson, 1962a). The yeast-like and mycelial form differ as regards their chemical composition and the structure of the cell wall (Bartnicki-Garcia & Nickerson, 1962b). Yeast-like growth of *Paecilomyces viridis* is not induced by changes in the cultivation temperature or by anaerobic conditions in submerse cultivation (Betina *et al.*, 1966). Growth

is partly inhibited in a N_2 and CO_2 atmosphere, but yeast-like cells are not formed (Baráthová & Betina, unpublished results). Antibiotics such as actinomycin D, azalomycin F, citrinin, cyanein, desertomycin, griseofulvin, patulin, rugulosin, trichothecin and viridin induce yeast-like growth of *Paecilomyces viridis*. Actinomycin D and trichothecin induce changes only after prolonged contact. Citrinin, cyanein and desertomycin, which release certain metabolites or pigments from cells (Betina & Baráthová, 1968; Baráth *et al.*, in press; Betina *et al.*, 1969) also induce branching and yeast-like growth in *Paecilomyces viridis*. Patulin releases anthocyanins from beetroot slices (Gäumann *et al.*, 1947). The polyene antibiotics filipin, flavofungin and nystatin, whose effect is associated with impairment of the permeability of cell membranes, do not induce changes in *Paecilomyces viridis*. Azalomycin F, however, which is also a polyene, but differs from the others by antibacterial effect (Sugawara, 1967), induces yeast-like growth of *Paecilomyces viridis* in relatively low concentrations. These findings indicate that impairment of permeability is not the only type of action needed for the induction of morphological changes, but that further interference in metabolism is required. It appears as though inhibitor-induced disturbances of normal metabolism can be manifested secondarily in morphological changes.

The plates will be found at the end of the issue.

References

Aytoun, R. S. C.: *The effects of griseofulvin on certain phytopathogenic fungi.* Ann. Botany (London) 20 : 297, 1956.

Bartnicki-Garcia, S., Nickerson, W. J.: *Induction of yeast-like development in Mucor by carbon dioxide.* J. Bacteriol. 84 : 829, 1962a.

Bartnicki-Garcia, S., Nickerson, W. J.: *Nutrition, growth and morphogenesis of Mucor rouxii.* J. Bacteriol. 84 : 841, 1962b.

Betina, V., Montagnier, L.: *Action de la cyanéine sur la synthèse des acides nucléiques et des proteines chez des cellules antimales et des protoplastes bactériens.* Bull. Soc. Chim. Biol. 48 : 194, 1966.

Betina, V., Baráthová, H.: *Citrinin-an inducer of permeability changes in Eremothecium ashbyi.* J. Antibiot. Ser. A 21 : 628, 1968.

Betina, V., Drouhet, E., Segretain, G.: *Action de la cyanéine in vitro sur des champignons pathogènes.* Ann. Inst. Pasteur 109 : 933, 1965.

Betina, V., Betinová, M., Kutková, M.: *Effect of cyanein on growth and morphology of pathogenic fungi.* Arch. Mikrobiol. 55 : 1, 1966.

Betina, V., Baráthová, H., Nemec, P., Baráth, Z.: *Permeability changes induced by desertomycin in fungal, animal and plant cells.* J. Antibiot. 22 : 129, 1969.

Blank, H., Taplin, D., Roth, F. J.: *Electron microscopic observations on the effects of griseofulvin on dermatophytes.* A. M. A. Arch. Dermatol. 81 : 667, 1960.

Brian, P. W., Curtis, P. J., Hemming, H. G.: *A substance causing abnormal development of fungal hyphae produced by Penicillium janczewskii Zal. I.* Trans. Brit. Mycol. Soc. 29 : 173, 1946.

Brian, P. W., Curtis, P. J., Hemming, H. G.: *A substance causing abnormal development of fungal hyphae produced by Penicillium janczewskii Zal. III.* Trans. Brit. Mycol. Soc. 32 : 20, 1949.

Drobnica, L., Betina, V.: *Effect of the antibiotic cyanein on the synthesis of nucleic acids and proteins by yeasts and bacteria.* Abstr. IXth Int. Congr. Microbiol., p. 215, Moscow, 1966.

El-Nakeeb, M. A., McLellan, W. L. Jr., Lampen, J. O.: *Antibiotic action of griseofulvin on dermatophytes.* J. Bacteriol. 89 : 557, 1965.

Gäumann, E., Jaag, O., Braun, R.: *Antibiotika als pflanzliche Plasmagifte.* Experientia 3 : 70, 1947.

Gilardi, G. L.: *Nutrition of systematic subcutaneous pathogenic fungi.* Bacteriol. Rev. 29 : 406, 1965.

Gundersen, K., Wadstein, T.: *Morphological changes and resistance induced in Saccharomyces pastorianus by the antibiotic cycloheximide.* J. Gen. Microbiol. 28 : 325, 1962.

Hall, G. E.: *Identification of baccatin A as an mixture of enniatins A and B.* Chem. and Ind. 1270, 1960.

Hessayon, D. G.: *Double action of trichothecin and its production in soil.* Nature 168 : 998, 1951.

Hütter, R., Keller-Schierlain, W., Nüsch, J., Zähner, M.: *Stoffwechselprodukte von Mikroorganismen.* 48. Mitt. Scopamycine. Arch. Mikrobiol. 51 : 1, 1965.

Isaac, I., Milton, J. M.: *Response of Verticillium species to griseofulvin.* J. gen. Microbiol. 48 : 65, 1967.

Kerridge, D.: *The effect of actidion and other antifungal agents on nucleic acid and protein synthesis*

in Saccharomyces carlsbergensis. J. gen. Microbiol. 19 : 497, 1958.

Klinkowski, M.: *Fungistatic antibiotics, especially polyenes and plant protection*. In: Recent advances in Botany p. 393, University of Toronto, 1961.

Mariat, F.: *Saprophytic and parasitic morphology of pathogenic fungi*. Symp. Soc. Gen. Microbiol., No. XIX, Microbiol Behaviour *in vitro* nad *in vivo*, p. 85, Cambridge Univ. Press, 1964.

Park, D., Robinson, P. M.: *Isolation and bioassay of a fungal morphogen*. Nature 203 : 988, 1964.

Pastan, I., Friedman, R. M.: *Actinomycin D: Inhibition of phospholipid synthesis in chick embryo cells*. Science 160 : 316, 1968.

Pressman, B. C.: *Induced active transport of ions in mitochondria*. Proc. Nat. Acad. Sci. U. S. 53 : 1076, 1965.

Robinson, P. M., Park, D.: *The production and quantitative estimation of a fungal morphogen*. Trans. Brit. Mycol. Soc. 48 : 561, 1965.

Robinson, P. M., Park, D.: *Citrinin-a fungistatic antibiotic and narrowing factor*. Nature 211 : 883, 1966.

Segretain, G., Drouhet, E., Mariat, F.: *Les mycoses et les champignons pathogènes*. Extrait du traité de biologie appliquée, p. 315, Librairie Maloine S. A. Paris, 1963.

Segretain, G., Fromentin, H., Destombes, P., Brygoo, E. R., Dodin, A.: *Paecilomyces viridis n. sp., champignon dimorphique agent d'une mycose généralisée de Chameleo lateralis Gray*. C. R. Acad. Sci. (Paris) 259 : 258, 1964.

Shemyakin, M. M.: *Chemistry of depsipeptide antibiotics*. Antimicrobial Agents and Chemotherapy – 1965, p. 962, American Society for Microbiology, Ann Arbor, 1966.

Sugawara, S.: *Mode of action of azalomycin F. Effect of azalomycin F on Candida albicans*. J. Antibiot. Ser. A 20 : 93, 1967.

Thomas, D. des S., Mullins, J. T.: *Role of enzymatic wall-softening in plant morphogenesis, Hormonal induction in Achlya*. Science 156 : 84: 1967.

EFFECT OF DATES OF PLANTING, VARIETIES AND SPACINGS ON OCCURRENCE OF INTERNAL BROWN SPOT IN POTATOES

*Mohammad Atta Shuja**

ABSTRACT

An experiment was conducted in 1967 at the Agricultural Research and Education Center in the Beqa'a plain, Lebanon, to determine the effect of three planting dates, two varieties and three spacings on occurrence of internal brown spot in potatoes.

The incidence of internal brown spot was markedly decreased with delay in planting. The small size tubers were least affected. The variety Arran Banner was highly susceptible to the disorder, but Patrones was almost unaffected.

INTRODUCTION

The potato (*Solanum tuberosum L.*) is one of th
It is also the leading vegetable crop. About 20.25 mil
potatoes in 1967 which produced about 27.04 million
It produces three to four times more food per unit area
also gaining importance as a feed and as a source of cl

In addition to high yielding variety, good cul
diseases are important factors, in getting higher yields.
affect the quantity and quality of the potato, internal
position. It is a symptomless physiological disorder w
by varieties, weather, cultural practices and size of the t

The present research work was done to find out
variety and spacing to get disease (internal brown spot)

SHUJA, M.A.

Effect of dates of plantin
on occurrence of internal

W. Pak. Jnl agric. Res.,

Ayub agric. Res. Inst.

Microfilm no. 156

REVIEW OF LITERATURE

Atanasoff (1) reviewed the work of Horne who appears to have reported this disease for the first time in 1911. Horne described it in two forms ; internal disease and streak. The disease at present is found in nearly all potato growing areas of the world and is known by various names, *e.g. Kringeringheid* in the Netherlands, *bunt fleckigkert* in Germany, sprain in England, internal brown fleck in South Africa, and

* Ayub Agricultural Research Institute, Lyallpur.

internal browning and internal brown spot in the U.S. Atanasoff (1) saw no visible symptoms on the outside of the tubers. He did not believe that the disease was a physiological disorder but thought that an organism, which could enter the tuber without leaving any distinctive sign at the site of entrance, was responsible for it. He also discounted the idea that lack of moisture was responsible for it and, in fact, suggested that an excess of moisture was a pre-requisite for occurrence of the disease in severe cases. Edmundson *et al.* (4) described the disease as dry brown spots scattered throughout the flesh of the tuber. The spots were comprised of dead cells free from bacteria and fungi. There were no definite foliage symptoms. They recommended that affected tubers should not be used for seed purpose as they did not produce strong plants. Wolcot and Ellis (9) reported that internal browning injury to potato tubers was more severe when plants were grown under conditions of artificially maintained moisture supply than when under drought conditions. Larson and Albert (7) reported internal necrosis as being entirely a malady of the growing tuber, there being no manifestations of the disorder in the aerial parts of the plants in the field during the growing season. They noted a considerable increase in the amount and severity of necrosis in tubers with advance in season and ... differences in responses of varieties as only one variety, ... n the trial showed complete resistance.

... it the occurrence of internal brown spot decreased with ... ry (2) reported that the occurrence of internal brown ... dates of planting, varieties, and the size of the tuber. ... the findings of Choudhry (2) except that he failed to find ... on the occurrence of internal brown spot.

... MATERIAL AND METHODS

... conducted in the Agricultural Research and Education ... an University of Beirut which is situated in the northern ... ion. The soil of the Center is clay in texture and has an ... per cent. The pH ranges from 8.0 to 8.4 with a high ... icating that the soil is highly calcarious in nature.

... rom end of February through September. The summer is generally dry and mild. Average temperatures rise gradually with the maximum occuring in the month of June. Rainfall declines sharply as the season advances and generally there is no precipitation in June, July, August and September. Meteorological data regarding average monthly air and soil temperature, rainfall, and relative humidity for the potato growing season during 1967 at the AREC are given in Table 1.

The plots were under Barley in 1966. A good seed bed was prepared in March as soon as the soil became suitable for cultivation. The plots were fertilized with 12 Kg nitrogen as ammonium sulphate and 20 Kg P_2O_5 as super phosphate per *dunum*. Plantings were done on March 13, April 12 and May 15.

1969
...anting, varieties and spacings
...rnal brown spot in Potatoes.
...s., 7 (1):32-37. [3 tables.
...Inst., Lyallpur.]
...5633

EFFECT OF DATES OF PLANTING, VARIETIES AND SPACINGS ON OCCURRENCE OF INTERNAL BROWN SPOT IN POTATOES

*Mohammad Atta Shuja**

ABSTRACT

An experiment was conducted in 1967 at the Agricultural Research and Education Center in the Beqa'a plain, Lebanon, to determine the effect of three planting dates, two varieties and three spacings on occurrence of internal brown spot in potatoes.

The incidence of internal brown spot was markedly decreased with delay in planting. The small size tubers were least affected. The variety Arran Banner was highly susceptible to the disorder, but Patrones was almost unaffected.

INTRODUCTION

The potato (*Solanum tuberosum L.*) is one of the major food crops of the world. It is also the leading vegetable crop. About 20.25 million hectares were planted under potatoes in 1967 which produced about 27.04 million metric tons all over the world (3). It produces three to four times more food per unit area on the basis of dry matter. It is also gaining importance as a feed and as a source of chemicals.

In addition to high yielding variety, good cultural practices and freeness from diseases are important factors, in getting higher yields. Among the many diseases which affect the quantity and quality of the potato, internal brown spot holds a significant position. It is a symptomless physiological disorder which seems to be greatly influenced by varieties, weather, cultural practices and size of the tuber (286).

The present research work was done to find out the optimum date of planting, variety and spacing to get disease (internal brown spot) free yields.

REVIEW OF LITERATURE

Atanasoff (1) reviewed the work of Horne who appears to have reported this disease for the first time in 1911. Horne described it in two forms ; internal disease and streak. The disease at present is found in nearly all potato growing areas of the world and is known by various names, *e.g. Kringeringheid* in the Netherlands, *bunt fleckigkert* in Germany, sprain in England, internal brown fleck in South Africa, and

* Ayub Agricultural Research Institute, Lyallpur.

internal browning and internal brown spot in the U.S. Atanasoff (1) saw no visible symptoms on the outside of the tubers. He did not believe that the disease was a physiological disorder but thought that an organism, which could enter the tuber without leaving any distinctive sign at the site of entrance, was responsible for it. He also discounted the idea that lack of moisture was responsible for it and, in fact, suggested that an excess of moisture was a pre-requisite for occurrence of the disease in severe cases. Edmundson *et al.* (4) described the disease as dry brown spots scattered throughout the flesh of the tuber. The spots were comprised of dead cells free from bacteria and fungi. There were no definite foliage symptoms. They recommended that affected tubers should not be used for seed purpose as they did not produce strong plants. Wolcot and Ellis (9) reported that internal browning injury to potato tubers was more severe when plants were grown under conditions of artificially maintained moisture supply than when under drought conditions. Larson and Albert (7) reported internal necrosis as being entirely a malady of the growing tuber, there being no manifestations of the disorder in the aerial parts of the plants in the field during the growing season. They noted a considerable increase in the amount and severity of necrosis in tubers with advance in season and tuber size. They observed differences in responses of varieties as only one variety, Triumph, out of 22 varieties in the trial showed complete resistance.

Bllison (5) reported that the occurrence of internal brown spot decreased with delay in planting. Choudhry (2) reported that the occurrence of internal brown spot was greatly influenced by dates of planting, varieties, and the size of the tuber. Hoque (6) later confirmed all the findings of Choudhry (2) except that he failed to find any effect of date of planting on the occurrence of internal brown spot.

MATERIAL AND METHODS

The present study was conducted in the Agricultural Research and Education Center (AREC) of the American University of Beirut which is situated in the northern part of the Beqa's plain, Lebanon. The soil of the Center is clay in texture and has an organic matter content of 2.2 per cent. The pH ranges from 8.0 to 8.4 with a high calcium carbonate content indicating that the soil is highly calcarious in nature.

The growing season is from end of February through September. The summer is generally dry and mild. Average temperatures rise gradually with the maximum occuring in the month of June. Rainfall declines sharply as the season advances and generally there is no precipitation in June, July, August and September. Meteorological data regarding average monthly air and soil temperature, rainfall, and relative humidity for the potato growing season during 1967 at the AREC are given in Table 1.

The plots were under Barley in 1966. A good seed bed was prepared in March as soon as the soil became suitable for cultivation. The plots were fertilized with 12 Kg nitrogen as ammonium sulphate and 20 Kg P_2O_5 as super phosphate per *dunum*. Plantings were done on March 13, April 12 and May 15.

Two potato varieties ; Arran Banner, a commonly grown variety in Lebanon which is highly susceptible to internal brown spot and Patrones, a promising variety were imported from Holland for plantation. The tubers were planted 15 cm, 25 cm and 35 cm apart within row and 75 cm distance was kept between the rows. Weeding was done as and when required.

The experimental design was split-split-plot and there were four replications. Planting dates were the main plots, varieties sub-plots and spacings were the sub-sub-plots. There were four rows in each sub-sub-plot. The plants of three plantings were harvested on August 22, August 29 and September 5 respectively. To avoid border effects two central rows were harvested. The end plants were also discarded. The data were analysed according to the method appropriate to the design according to Snedecor and Cochran (8).

The commonly grown variety (Arran Banner) and the standard practice of plantation (March 13) and spacing (25 cm) were taken as check.

RESULTS AND DISCUSSION

Planting date had a marked effect on the occurrence of internal brown spot in potatoes (Table 2). Least affected tubers were produced with May 15 planting while the earliest planting produced the most affected tubers. This highly significant difference can be (Dunum= 1,000 sq. meter or about 1/4 acre) attributed to the comparatively lower temperature at the time of tuber maturation with delayed planting. Hoque (6) found no real difference due to planting date but Choudhry (2) and Ellison and Jacob (5) reported positive results. Arran Banner was highly significantly more susceptible than Patrones. Interaction of planting date with varieties was also found to be significant. Arran Banner produced the greatest number of affected tubers when planted on March 13, less with April 12 planting, and least with the May 15 planting. Patrones produced completely healthy tubers with the last date of planting.

TABLE 1

The Average Monthly Air and Soil Temperature in Degree Centigrade, Rainfall in Millimeter, and Relative Humidity in Per cent at the Agricultural Research and Education Centre from March Through September, 1967

	March	April	May	June	July	August	September
Air temperature							
Mean maximum	10.94	16.65	21.79	26.05	29.27	26.60	26.50
Mean minimum	0.95	3.47	8.43	9.68	12.16	12.25	10.00
Soil temperature							
Sod 10 cm.	8.65	13.78	18.84	21.27	23.82	24.64	21.69
Bare earth 10 cm deep.	8.90	15.25	20.62	23.98	26.18	26.24	22.96
Rainfall	167.1	20.5	34.5	0.0	0.0	0.0	0.0
Relative humidity							
Mean maximum	88.1	77.7	76.4	64.1	60.2	63.3	75.1
Mean minimum	54.8	39.5	36.8	32.4	30.8	34.1	37.9

TABLE 2

Effect of Planting Date, Variety and Spacing on Percentage Incidence of Internal Brown Spot in Marketable Potato Tubers During 1967

Planting date	Varieties	Spacings 15 cm	25 cm	35 cm	Mean	Mean of planting date
March 13	Arran Banner	73.75	86.25	88.75	82.91	41.87
	Patrones	1.25	0.00	1.25	0.83	
	Mean	37.50	43.12	45.00		
April 12	Arran Banner	67.50	68.75	61.26	65.83	33.12
	Patrones	0.00	0.00	1.25	0.41	
	Mean	33.75	34.37	31.25		
May 15	Arran Banner	38.75	46.25	42.50	42.50	21.25
	Patrones	0.00	0.00	0.00	0.00	
	Mean	19.37	23.12	21.25		
Mean Arran Banner		60.00	67.08	64.16	63.74	
Mean Patrones		0.41	0.00	0.83	0.41	
Mean Spacings		30.20	33.54	32.50		

	LSD (5%)	LSD (1%)
Planting date.	1.76	2.67
Varieties.	1.19	1.61
Planting date × varieties.	2.90	3.93
All others.	N.S.	N.S.

The incidence of internal brown spot in individual tubers was greatly influenced by their sizes (Table 3). Tubers of large and medium sizes were affected to a much greater extent than those of small size. This highly significant difference can be attributed to the differences in the maturity of the tubers at harvesting time. The occurrence of internal brown spot generally appears during the maturing stage of tubers. Small tubers are physiologically immature at the time of harvest so are less affected. The results are in agreement with those obtained by Choudhry (2), Hoque (6) and Larson and Alber (7).

The interaction between varieties and sizes was also found to be significant at the 1% level (Table 3). Arran Banner produced the greatest number of affected tubers in

large size, the next greatest in the medium size, and the least in the small size. Patrones produced a very small number (0.83%) of affected tubers in the large size but none in the medium or small sizes.

TABLE 3

Effect of Tuber Size on Percentage Incidence of Internal Brown Spot in Potatoes During 1967

Varieties	Size of tubers			Mean of varieties
	Large	Medium	Small	
Arran Banner	77.22	48.88	9.16	45.08
Patrones	0.83	0.00	0.00	0 27
Mean of sizes	39.02	24.44	4.56	

	LSD (5%)	LSD (1%)
Varieties	7.09	13.02
Sizes	4.03	5.64
Varieties × sizes.	9.43	13.20

SUMMARY

A field experiment was laid-out to determine the effects of date of planting. varieties and spacings on occurence of internal brown spot in potatoes. This study was carried out at Agricultural Research and Education Center of the American University of Beirut situated in the northern Beqa'a plain of Lebanon. Two varieties, Arran Banner and Patroness, were planted at three spacings of15 cm, 25 cm, ann 35 cm on March 13, April 12 and May 15.

Delay in planting decreased the occurrence of internal brown spot; however, its occurance was not effected by spacing. Patrones was practically free of this physiological disorder as only 0.41% of the tubers were affected in the first two plantings and none in the last. In general, large tubers showed a much greater susceptibility to internal brown spot than did the small ones.

It can be concluded that selecting a resistant variety is the most practical method to over come the losses from internal brown spot.

REFERENCES

1. Attanasoff, D. 1962 Sprain or internal brown spot of potatoes. Phytopath. 16, 711. 1926

2. Choudhry, A.H, 1963 Effect of planting dates on yield, internal brown spot and other characteristics in potatoes. M.S. Thesis. American University of Beirut. Beirut, Lebanon.

3. Cramer, H.H. 1967 *Pflanzenschutz nachrichten*. Farbenfavriken Bayer Agency. Leverkusen.

4. Edmundson, W.C., L.A. Schall, and B.J. Landis. 1951 Potato growing in the western states. U.S.D.A. Farmer Bull. 2034.

5. Ellison, J.H., and W.C. Jacob. 1952 Internal browning of potatoes as affected by date of planting and storage. Amer. Potato J. 29, 241.

6. Hoque, M.M. 1964 Effect of planting date and varieties on yield, internal brown spot and growth characteristics of potatoes. M.S. Thesis. American University of Beirut. Beirut, Lebanon.

7. Larson, R.H., and A.R. Albert. 1945 Physiological internal necrosis of potato tubers in Wisconsin. J. Agr. Res. 71, 487.

8. Snedecor, W.G., and W.G. Cochran. 1965 1956 *Statistical Methods*. The Iowa State University Press, Ames, Iowa, U.S.A.

9. Wolcot, A.R., and N.K. Ellis. Associated forms of internal browning of potato tubers in Northern Indiana. Amer. Potato J. 33, 343.

8.	Kamal, M.	1958	Studies on the physiology of root rot of cotton in Pakistan. II, protopectinase production by Rhizoctonia spp. as influenced by nitrogen sources and its role in disease syndrome. The Pak. Cottons. 11 (2) : 13-16.
9.	Kendrick, J.B.	1933	Seedling stem blight of field beans caused by Rhizoctonia bataticola at high temperatures. Phytopath. 23: 949.
10.	Livingston, J. E.	1945	Charcoal rot of corn and sorghum. Res. Bull. Neb. Agri. Expt. Sta. 136 (32) : 5.
11.	Luthra, J. C & Vasudeva, R. S.	1938	Studies on the root rot disease of cotton in the punjab. Ind. J. Agri. Sci. 8 : 727—734.
12.	Narasimhan	1932	Report on the work done in the Mycological Section. ...A.M.

... Patho-
...D45.

... a and
...unjab.

...opath.

1968

PERWAIZ, M.S., MOGHAL, S.M. & KAMAL, M.

Studies on the chemical control of Alternaria fruit rot of Chillies.

W. Pak. Jnl agric. Res., 6 (4):87-91. [2 tables. Agric. Res. Inst., Tando Jam.]

Microfilm no. 15634

STUDIES ON THE CHEMICAL CONTROL OF ALTERNARIA FRUIT ROT OF CHILLIES

*M. S. Perwaiz, Sardar M. Moghal and M. Kamal.**

INTRODUCTION

Capsicum frutesence L. (Giant chillies) is an important vegetable which is mostly used for salads, stuffings and pickles and has many medicinal properties. It is grown as a cash crop at several places of Hyderabad region but fruit rot, a serious disease caused by *Alternaria tenuissima* (Nees ex Fr.) Wiltshire, stands in the way of its successful cultivation. Preliminary investigations on the disease and the physiology of causal fungus revealed that the disease is destructive in nature, Kamal and Tahiruddin, (1968). Disease mostly appears on the developing fruits which become unfit for consumption. It is not uncommon to observe as much as 20% infections of this disease under natural field conditions.

Some work has been reported earlier on the chemical control or rot of chillies. Chaudhary (1) claimed Perenox, Dithane and Bordeaux mixture as the best spray fungicides for checking Colletotrichum rot of chillies, *Capsicum annum L.* Toxic effect of some copper and organic fungicides against some *Alternaria* spp. was studied by Misra and Pal (1965). Since no work of this nature has so far been conducted on the control of Alternaria rot of giant chillies, efficacy of some spray fungicides was studied against the disease as it exists in this region.

MATERIAL AND METHODS

These investigations were conducted in the Plant Pathology Section, Agricultural Research Institute, Tandojam during 1966 and 1967. Experiment was laid-out using a 5×5 Latin square design. 24 healthy seedlings of "Chinese Giant" variety were transplanted on 6 ridges in each plot which measured 9×9 ft.

Inoculation of Fruits : Causal fungus was isolated from rotted fruits and was multiplied on 2 per cent Potato-dextrose agar plates by incubating them at 26-28°C for 10 days. Water suspension of fungal conidia was prepared using the usual procedures, and on microscopic examination each drop of inoculum contained 6-10 conidia of *A. tenuissima*. Fruits in each plot were inoculated with the fungal suspension by means of a syringe in the morning hours. Disease was thus produced under artificial conditions on inoculation.

* Agricultural Research Institute, Tando Jam.

Application of Fungicides : Four fungicides as shown below were tested against the disease. Laboratory studies were also conducted on the toxicity of these fungicides against the fungus using Food Poisoned Technique, Horsfall (1956).

Chemical	*Concentration*	*Active Ingredient*	*Source*
Copper Sandoz	2 1b/100 gal.	50% metallic copper as Cuo.	Sandoz
Bordeaux mixture	4 : 4 ; 50	A complex of copper hydroxide and calcium sulphate.	Local
Dithane M-45	2 1b/100 gal.	Zinc-manganese ethylene bisdithiocarbamate.	Rohm & Haas
Makrolin	0.2 %	An indigenous product of chlorinated hydrocarbon.	P. C. S. I. R. Karachi.

Two days after incoculation fist spraying with respective fungicides was made. Control consisted of inoculated but unsprayed plots. Spraying was repeated at an interval of 15-20 days, and in all the crop received three applications. Completely healthy and diseased fruits were harvested and counted to calculate per cent disease incidence and yield. Statistical methods appropriate to the design of experiment were applied for the analysis of the data.

EXPERIMENTAL RESULTS

Growth behaviour of *A. tenuissima* as influenced by respective fungicides is shown in Table I.

TABLE I

Effect of Fungicides on Growth of A. tenuissima

Treatment	Linear Colony Growth mm.
Copper sandoz	4.0
Bordeaux mixture	5.0
Dithane	8.0
Makrolin	16.0
Control	32.0
L. S. D. at P=0.05	3.5
L. S. D. at P=0.01	5.5

It can be observed from the above data that the fungicides tested in culture medium checked linear growth of *A. tenuissima* which in case of control was maximum *i.e.* 32 mm. after 10 days. Copper Sandoz and Bordeaux mixture, followed by Dithane, gave good inhibition. Markolin showed only 50 % inhibition of the growth of the fungus.

Data showing the effect of fungicides on the incidence of fruit rot and yield of chillies are presented in Table II.

TABLE II

Effect of Fungicides Against Alternaria Rot of Chillies

	% Disease Incidence			Yield	lbs/Plot	
Treatments	1966	1967	Mean	1966	1967	Mean
Copper Sandoz	4.3	7.7	6.0	19.5	12.3	15.9
Bordeaux mixture	8.5	9.5	9.0	17.1	11.9	14.5
Dithane	13.1	12.4	12.7	14.8	10.8	12.8
Makrolin	13.3	20.3	16.8	12.1	10.9	11.5
Control	24.3	27.1	25.7	10.8	9.4	10.1
L. S. D. at P=0.05	3.12	3.42	—	3.21	0.62	—
L. S. D. at P=0.01	4.38	4.80	—	4.51	0.86	—

Incidence of fruit rot of chillies in the unsprayed plots in 1966 and 1967 was 24.3 and 27.1% respectively with an average of 25.7 %. There was a significant reduction in disease incidence when the inoculated plots were sprayed with fungicides. Among the treatments Copper sandoz gave the lowest disease incidence *i.e.* 6 %. This was followed by Bordeaux mixture but with no significant different between these two treatments. Sprayed plants produced higher yields over control. Copper sandoz and Bordeaux mixture again gave significantly higher yields as compared to other fungicides.

DISCUSSION

Preliminary investigations had indicated that the source of infection of fruit rot of chillies is external. It appeared that a thorough coverage of developing fruits with fungicides would help in controlling the disease and increasing the yield. The present work was, therefore, undertaken to investigate the effect of four spray fungicides against the fungus and the disease caused by it.

It was found that the fungicides tested could inhibit the growth of *Alternaria tenuissima* when incorporated in the culture medium and grown at an optimum temperature of 26-28°C. Inhibition of the linear colony growth was more pronounced in case of copper Sandoz and Bordeaux mixture followed by Dithane. These findings are in general agreement with those of Misra and Pal (1965) who have shown the effectiveness of copper and organic fungicides such as Bordeaux mixture and Dithane against some *Alternaria* spp. under laboratory conditions in India. Makrolin allowed sufficient growth of the fungus.

Spraying controlled the disease to a great extent and helped in getting higher yields of chillies over control. Best results were obtained with Copper Sandoz and Bordeaux mixture. These were followed by Dithane. In general there was a close relationship between toxic effect of fungicides, on the fungus, disease incidence and yield. These studies indicated that copper fungicides are more effective against fruit rot of giant chillies than the others. Chaudhary (1) also reported a good control of Colletotrichum rot of chillies by copper fungicides as Perenox and Bordeaux mixture. Fungicides tested under the local conditions did not exhibit any toxic or adverse effect on plants or fruits. Three sprayings in the season proved to be sufficient for the control of the desease. Studies on relative economics revealed that the control of fruit rot of chillies with an effective agent can bring the growers an increase in yield of the order of 15 to 20 % resulting in substantial additional income.

SUMMARY

Four spray fungicides *viz.* Copper Sandoz, Bordeaux mixture, Dithane M-45 and Makrolin were tested for their efficacy against fruit rot of giant chillies caused by *Alternaria tenuissima*. Disease was reproduced in the field under artificial conditions of inoculation before spraying operations. In laboratory studies Copper Sandoz, Bordeaux mixture and Dithane when incorporated in the culture medium inhibited the growth of fungus. Fungicidal sprays gave less disease incidence and produced higher yields over control. Best results were obtained with Copper Sandoz and Bordeaux mixture which reduced the infection to 6 and 8 % respectively as against 25.7 % in control. Dithane also produced good results.

ACKNOWLEDGEMENT

Thanks are due to Mr. M. M. Siddiki, Director, Agricultural Research Institute, Tandojam for going through the manuscript and giving useful suggestions.

REFERENCES

1. Chaudhary, S. 1957 Studies on the Development of Fruit Rot of Chillies. Ind. Phytopath. 10 : 55-62.

2.	Horsfall, J. S.	1956	Principles of Fungicidal Action, Chronica Botanica Co. Publ. Waltham Mass, U. S. A.
3.	Kamal, M and Tahiruddin.	1968	Studies on Alternaria Rot of Chillies in Pakistan and the Physiological Behaviour of the Causal Fungus. (Manuscript).
4.	Misra, A. P., and T. B. Singh.	1965	Toxicity, Physiological and Biological Spectum of Certain Copper and Organic Fungicides Ind. Phytopath. 18 : 181—190.

STUDIES ON SEED BORNE MICROFLORA AND THE EFFECT OF THE SEED TREATMENT OF RICE

*Ghulam Rasool Solangi, M. Kamal and R. M. Abbasi**

INTRODUCTION

Rice is a staple crop of Pakistan next to wheat. It occupies an area of about 2 million acres in Hyderabad and Khairpur Divisions.

This crop suffers from many diseases in all rice gı which the most important are blast (*Piricularia-oryzae*) aı *porium-oryzae*).

The main fungi reported from rice seeds are spı *Helminthosporium, Fusarium, Penicillium, Curvularia, Piric* and Corbetta, (2) and *Trichoconis padwickii, Curvularia l* *Helminthosporium-oryzae* and *Nigrospora-oryzae* Padma *lunata* and *Helminthosporium-oryzae* (Hingorani and Prasac made from different varieties of rice seeds revealed the prese *Curvularia Helminthosporium, Chaetomium, Nigrospora, Stei* *porium Aspergillus* and *Mucor* Mirza and Ali, (4) ; Bajwa

Preliminary studies made by the author on the seed cated relatively high percentage of seed infestation with var therefore, been made to identify the main organisms associated with rice seed and study the comparative efficacy of different fungicides against these organisms.

SOLANGI, G.R., KAMAL, M. a

Studies on seed borne micr
seed treatment of Rice.

W. Pak. Jnl agric. Res.,
Agric. Res. Inst.,

Microfilm no. 15

MATERIAL AND METHODS

Rice seeds of seven different varieties were collected from Agricultural Research Station, Dokri. All these samples were six months old. Standard International Rules for seed testing was followed throughout these studies (I-Rules, 1959).

Three hundred seeds were taken from each of the seven varieties, they were surface disinfected for 2-3 minutes in mercuric chloride 0.1% and dried between blotters ; 5 seeds were plated out in each dish containing 2% P.D.A. and then incubated at 28°C for 8 days. Observations were taken when most of the fungi were grown.

*Agricultural Research Institute, Tando Jam.

Seed treatment experiment was conducted at Agricultural Research Station, Dokri. For this a variety Bengalo which was found most susceptible to seedling blight was selected. The fungicides used were :

1. Agrosan G. N. — Tolyl mercuric acetate (I. C. I.) 2 oz/30 seers of seed.
2. Ceresan — Ethyl mercury phosphate (Bayer) 2 oz/30 seers of seed.
3. Granosan M. — Ethyl mercuric P Toluene Sulphonanilide. ½ oz/30 seers of seed.

Paddy seeds of Bengalo variety were disinfested with 0.1% mercuric chloride solution and were infested artificially with a mixture of nine seed borne fungi comprising *Trichoconis padwickii, Cochlibolus spicifer, Curvularia-verruculosa, Chaetomium globosum,* ... *lternaria spp., Fusarium spp.*, and *Xanthomonas sp.* ... ve fungi were then treated with each of the above ... with out seed borne fungi served as control. One ... ient were transplanted in 1/40th acre plots. The ... l plots of 1/40th of an acre, with four replications. ... paddy were recorded.

1968
M. & ABBASI, R.M.
microflora and the effect of the
e.
es., 6 (4):92-98. [3 tables.
nst., Tando Jam.]
5635

...IENTAL RESULTS

... from seven different varieties of rice, viz, Dokri ... Kangni × Torh, Sonhori Sugdasi, Kangni 27 and ... cting the seeds was determined by the frequency of ... I).

...tion

The effect of main isolates obtained from rice seeds on germination was determined. Seeds of susceptible variety (Bengalo) were surface sterlized, immersed in spore suspension of desired fungus for ½ hour Hingorani and Prasad, (3) and were sown in sterlized soil in pots. A total number of 50 seeds in each treatment was used.

STUDIES ON SEED BORNE MICROFLORA AND THE EFFECT OF THE SEED TREATMENT OF RICE

*Ghulam Rasool Solangi, M. Kamal and R. M. Abbasi**

INTRODUCTION

Rice is a staple crop of Pakistan next to wheat. It occupies an area of about 2 million acres in Hyderabad and Khairpur Divisions.

This crop suffers from many diseases in all rice growing regions of the world of which the most important are blast (*Piricularia-oryzae*) and brown spot (*Helminthosporium-oryzae*).

The main fungi reported from rice seeds are species of *Epicoccum, Alternaria, Helminthosporium, Fusarium, Penicillium, Curvularia, Piricularia* and *Bacteria* (Baldacci and Corbetta, (2) and *Trichoconis padwickii, Curvularia lunata, Ophiobolus miyabeanus, Helminthosporium-oryzae* and *Nigrospora-oryzae* Padmanabhan, (6) and *Curvularia lunata* and *Helminthosporium-oryzae* (Hingorani and Prasad, (3). In Pakistan isolations made from different varieties of rice seeds revealed the presence of species of *Alternaria, Curvularia Helminthosporium, Chaetomium, Nigrospora, Stemphylium, Fusarium, Macrosporium Aspergillus* and *Mucor* Mirza and Ali, (4) ; Bajwa and Kausar, (1).

Preliminary studies made by the author on the seed borne microflora of rice indicated relatively high percentage of seed infestation with various fungi. An attempt has, therefore, been made to identify the main organisms associated with rice seed and study the comparative efficacy of different fungicides against these organisms.

MATERIAL AND METHODS

Rice seeds of seven different varieties were collected from Agricultural Research Station, Dokri. All these samples were six months old. Standard International Rules for seed testing was followed throughout these studies (I-Rules, 1959).

Three hundred seeds were taken from each of the seven varieties, they were surface disinfected for 2-3 minutes in mercuric chloride 0.1% and dried between blotters ; 5 seeds were plated out in each dish containing 2% P.D.A. and then incubated at 28°C for 8 days. Observations were taken when most of the fungi were grown.

---

*Agricultural Research Institute, Tando Jam.

Seed treatment experiment was conducted at Agricultural Research Station, Dokri. For this a variety Bengalo which was found most susceptible to seedling blight was selected. The fungicides used were :

1. Agrosan G. N. — Tolyl mercuric acetate (I. C. I.) 2 oz/30 seers of seed.
2. Ceresan — Ethyl mercury phosphate (Bayer) 2 oz/30 seers of seed.
3. Granosan M. — Ethyl mercuric P Toluene Sulphonanilide. ½ oz/30 seers of seed.

Paddy seeds of Bengalo variety were disinfested with 0.1% mercuric chloride solution and were infested artificially with a mixture of nine seed borne fungi comprising *Trichoconis padwickii, Cochlibolus spicifer, Curvularia-verruculosa, Chaetomium globosum, Nigrospora state, Aspergillus spp., Alternaria spp., Fusarium spp.*, and *Xanthomonas sp.* The paddy seeds infested with the above fungi were then treated with each of the above three fungicides. Paddy seeds infested with out seed borne fungi served as control. One month old seedlings of each treatment were transplanted in 1/40th acre plots. The experiment was designed in randomized plots of 1/40th of an acre, with four replications. Data on disease incidence and yield of paddy were recorded.

EXPERIMENTAL RESULTS

1. Seed Mycoflora

Isolations were made separately from seven different varieties of rice, viz, Dokri Basmati, Sonhari Kangni, Jajai 77, Kangni × Torh, Sonhori Sugdasi, Kangni 27 and Bengalo. The Percentage of fungi infecting the seeds was determined by the frequency of their occurrence on isolations. (Table I).

2. Effect of Seed Isolates on Germination

The effect of main isolates obtained from rice seeds on germination was determined. Seeds of susceptible variety (Bengalo) were surface sterlized, immersed in spore suspension of desired fungus for ½ hour Hingorani and Prasad, (3) and were sown in sterlized soil in pots. A total number of 50 seeds in each treatment was used.

TABLE I

Organsims Associated with Seeds of Different Rice Varieties (Frequency of Isolates in Percentage)

S. No.	Organism	Kangni 27	Sonhari Sugdasi	Sonhari Kangni	Bengalo	Dorki Basmati	Jajai 77	Kangni × Torh	Combined Frequency
1.	*Xanthomonas sp.*	61.0	31.5	43.0	64.5	59.0	51.0	56.0	52.3
2.	*Trichoconis padwickii*	31.0	41.4	10.7	32.5	31.0	35.0	36.0	31.1
3.	*Cochliobolus spicifer* (*Helminthosporium-oryzae*)	2.5	3.4	—	1.0	3.0	—	3.5	1.9
4.	*Curvularia verruculosa*	1.5	3.5	—	—	2.0	—	2.5	1.4
5.	*Chaetomium globosum*	2.5	—	—	—	—	1.5	—	0.6
6.	*Nicrospora state*	1.5	—	3.6	—	—	2.5	2.0	1.3
7.	*Aspergillus* Spp.	—	20.2	39.1	1.0	5.0	10.0	—	10.7
8.	*Alternaria* Spp.	—	—	3.6	—	—	—	—	0.6
9.	*Fusarium* Spp.	—	—	—	1.0	—	—	—	0.1

It is evident from these large scale isolations that different varieties yielded different fungi with varied frequencies. However, *Xanthomonas* sp. *Trichoconis padwickii*, *Cochliobolus spicifer*, *Curvularia verruculosa*, *Nigrospora State* and *Aspergillus* were predominnatly associated with rice seeds. Identity of the main isolates was confirmed by reference to Commonwealth Mycological Institute, Kew.

TABLE II

Effect of Seed Isolates on Germination

S. No.	Isolate	Germination percentage
1.	*Xanthomanas* sp.	70
2.	*Trichoconis padwickii*	73
3.	*Cochliobolus spicifer*	80
4.	*Curvularia verruculosa*	87
5.	*Nigrospora state*	85
6.	*Aspergilius* Spp.	76
7.	Control	90

It is clear from the above Table that associated organisms reduced the germination percentage of seed in varying degrees. *Xanthomonas* sp. were found to be most harmful followed by *Trichoconis padwikii*, *Aspergillus* Spp. and *Cochliobolus spicifer*.

Since these findings strongly indicated the harmful effect of associated organisms on seeds, experiments were laid-out to test the efficacy of certain recommended seed treating fungicides on disease incidence and yield of crop.

3. Effect of Seed Treatment on Yield and Helminthosporium Leaf Spot Incidence of Rice.

The experiment was conducted for three years 1964-66. Yield data and disease incidence were recorded every year. Observations on the incidence of the disease were taken on 25 plants in each treatment, selected at random. The results are given in Table III.

TABLE III

Yield Data and Disease Incidence in Bengalo Variety of Rice

Year	Agrosan G.N.	Ceresan	Granosan M.	Control	Significane	
					L.S.D at P=.05	L.S.D at P=.01
1964						
Yield in mds/acre	27.9	31.5	25.2	26.1	2.01	2.89
Disease %	5.0	3.0	5.5	7.0	1.13	1.62
1965						
Yield in mds/acre	27.3	30.4	27.1	27.1	1.78	2.58
Disease %	6.0	4.0	6.0	9.0	1.01	1.46
1966						
Yield in mds/acre	20.9	22.4	20.8	19.1	0.80	1.80
Disease %	7.5	5.0	7.5	15.0	1.62	2.32

It can be assumed from the above data that in all the three years, treated seeds in general produced higher yield of paddy over untreated seeds which ranged from 3-5 mds. on an average. Of the fungicides tried, best results were obtained with Ceresan M which proved to be significantly better than others.

It was further observed that with the increase in disease intensity there was a corresponding decrease in the yield.

DISCUSSION

Isolations made from seven different Rice varieties i.e. Dokri Basmati, Sonhari Kangni, Jajai 77, Kangni × Torh, Sonhari Sugdasi, Kangni 27 and Bengalo showed that although all the seeds looked clean and healthy yet they were contaminated by various fungi. The fungi isolated from above varieties were *Cochliobolus spicifer* (*Helminthosporium-oryzae*), *Trichoconis padwickii*, *Curvularia verruculosa*, *Chaetomium globosum*, *Nigrospora state*, *Aspergillus* spp., *Alternaria* spp., *Fusarium* spp., and *Xanthomonas* sp. Among these *Xanthomonas* sp., *Trichoconis padwickii*, *Cochliobolus spicifer*, *Curvularia verruculosa*, *Nigrospora state* and *Aspergillus* spp. were mostly predominant. These results are in conformity with those of Mirza and Ali (1960) who isolated species of *Alternaria*, *Curvularia*, *Helminthosporium*. *Chaetomium*, *Nigrospora*, *Stemphylium*, *Fusarium*, *Macrosporium*, *Aspergillus* and *Mucor* from paddy varieties, i.e. Kangni 27, Motia, Basmati, Jajai 77, Bengalo and Sonhari Kangni. Working with rice varieties grown in the former Bunjab area, Bajwa and Kausar (1) reported the occurrence of various fungi on rice seeds, but in their studies *Curvularia* followed by *Helminthosporium* spp., was found to be the most predominent and *Xanthomonas* sp. was not reported. Similar organisms have been found to be associated with rice seeds as reported by workers from other countries, *viz.*, Padmanabhan (6) from India, Baldacci and Corbetta (2) from Italy, Neergaard and Saad (5) from Denmark. It is interesting to note that work with different varieties and under entirely different conditions in Italy *Bacteria* were obtained in the highest frequency by Baldacci and Corbetta (2) and the fungus *Trichoconis padwickii* was reported to be occurring in the highest frequency by padmanabhan (6) in India. These observations indicate the importance of conducting such studies under different conditions.

Some of the fungi associated with rice seeds have already been reported causing various diseases, *viz.* *Helminthosporium-oryzae* and *Curvularia* spp. (causing seedling-blight) Hingorani and prasad (3), *Nigrospora-oryzae* (causing minute leaf spot) Thompson (1936).

These studies show that rice seeds act as active carriers of these pathogens in addition to harbouring harmful fungi which may cause seed rot and impair germination. The reduction of germination by associated fungi in respect or rice seeds was also observed by Padmanabhan (6) and Bajwa and Kausar (1).

The effect of fungicides on germination, disease incidence and yield revealed that fungicidal treatment increased germination, reduced incidence of disease and also increased the yield. Amongst the three fungicides tested Ceresan proved to be the best in efficacy during the three years of trials. Ceresan was also found to be effective in experiments conducted by Bajwa and Kausar (1) on rice seed treatment. Somewhat similar reduction in disease incidence by the application of fungicides was observed by Hingorani and prasad (3) although they did not test the efficacy of these fungicides in increasing yield in the field.

In conclusion, it may be said that the treatment of the rice seeds with Ceresan can be recommended for getting increased yields of rice under conditions prevailing in South West Pakistan.

SUMMARY

1. *Cochliobolus spicifer*, *Trichoconis padwickii*, *Curvularia verruculosa*, *Chaetomium globosum*, *Nigrospora state*, *Aspergillus* spp., *Alternaria* spp., *Fusarium* spp. and *Xanthomonas* sp. have been found to be associated with different varieties of rice seeds. Amongst these *Xanthomonas* sp., *Cochliobolus spicifer*, *Trichoconis padwickii* and *Aspergillus* spp. were observed in higher frequencies and were most effective in reducing germination of the seed.

2. Among the three fungicides tested, Ceresan M proved to be the best in improving germination, minimising disease incidence and increase yield.

ACKNOWLEDGEMENT

Thanks are due to Mr. M.M. Siddiki, Director of Research, Agricultural Research Institute, Tandojam for extending necessary facilities in connection with this work and the Director Commonwealth Mycological Institute, Kew for help in confirming the identity of micro-organisms associated with rice seed.

REFERENCES

1. Bajwa, M.N and Kausar, A.G. 1965 Fungi occurring on paddy seeds and their control. Pak. Jour. Agric. Sci. 2 : 7-20.

2. Baldacci. E. and Corbetta, G. 1950 Researches on the microflora of rice after storage and under experimental condition. Reprinted from II Riso, Mar. 1964. Publ. Its Patrol. veg. Univ., Milano 1964.

3. Hingorani, M.K. and Parasad, N. 1951 Seedling blight in Sind. Indian Phytopath. 4 : 60-83.

4. Mirza, R. and Ali, S.B. 1960 Notes on seed borne fungi of Pakistan. Proceedings of the 12th Pak. Science Conference. Hyderabad. part III Abstract. pp: A 47-48.

5.	Neergaard, P. and Saad, A.	1962	Seed health testing of rice. Indian Phytopath. 15: 85-111.
6.	Padmanabhan, S.Y	1949	Occurrence of fungi inside rice kernels. Curr. Sci. 18: 442—443.
7.	Thompson, A.	1936	Reprint of the Division of Mycology Dep. Agric. Malaysia, 1935, pp: 64-66.
8.	Anonymous	1959	International Rules for seed testing Proc. Int. seed test. A. SS. 24: 565—577.

SEASONAL VARIATION IN THE PROTEIN CONTENT OF BUFFALOE'S MILK

*Muhammad Aftab Khan**

INTRODUCTION

Recent advances in the field of proteins, particularly those of animal origin prompted a study to determine the effect of season on the protein content of buffaloes milk. Milk proteins by virtue of their favourable balance of essential amino acids have the highest biological value. Very little information is available in the literature relative to factors such as season calving variations in the protein content of buffaloes milk. Individual variations within buffaloes should be established if attempts are made to select towards high protein production. A study was thus conducted to determine the effect of season on the protein content of buffalloes milk.

REVIEW OF LITERATURE

Overman *et al.* (9) observed in Brown Swiss cows that the average protein percentage was low in summer month and high in winter months. Walts *el al.* (13) remarked that the seasonal effect were of smaller magnitude than those of stage of lactation and crude protein and casein contents rose to a peak in May-June, and again in September. Lowest values occurred from January to March. Nickerson (8) observed that protein contents of milk varied significantly ($P \angle 0.05$) with season. Higher values were recorded in November, December and January (winter season) and lowest in May, June and July (summer season). Paul and Mallan (10) Studied the milk composition of Indian animals (cows and buffaloes) and found that Solid-not-fat (SNF) reached to its higher peak in November and December and this level was maintained during winter months. A gradual fall in S.N.F. content was noted which reached its lowest level in May-June. Slight improvement occurred in June-July with the onset of rains and again in August-September a slight decrease was noted. Needham (7) observed that with the onset of summer the S. N. F. level fell rapidly, remained low until early winter till the green fodder was available and then rose steadily until the beginning of summer.

The S. N. F. contents of Murrah buffaloes were highest (9.97 per cent) in winter, decreased in spring and summer (9.09 and 9.86 per cent) respectively. and were lowest (9.73 per cent) in autumn National Dairy Research Institute, Karnal, (6). Kin *el al.* (4) in Japan found that protein content of milk during winter months was significantly higher

*West Pakistan Agricultural University, Lyallpur.